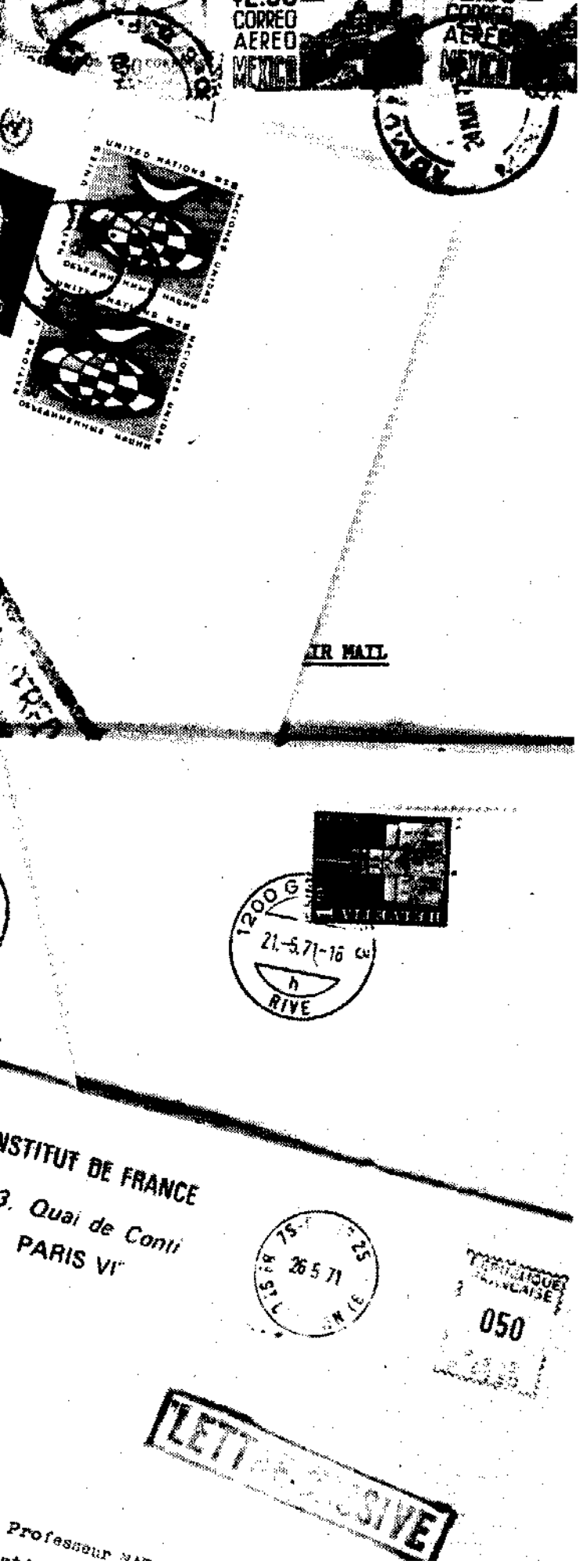
LES CAHIERS DE L'INSTITUT DE LA VIE

prix de l'institut de la vie

1972 nos 32, 33, 34
1973 nos 35, 36

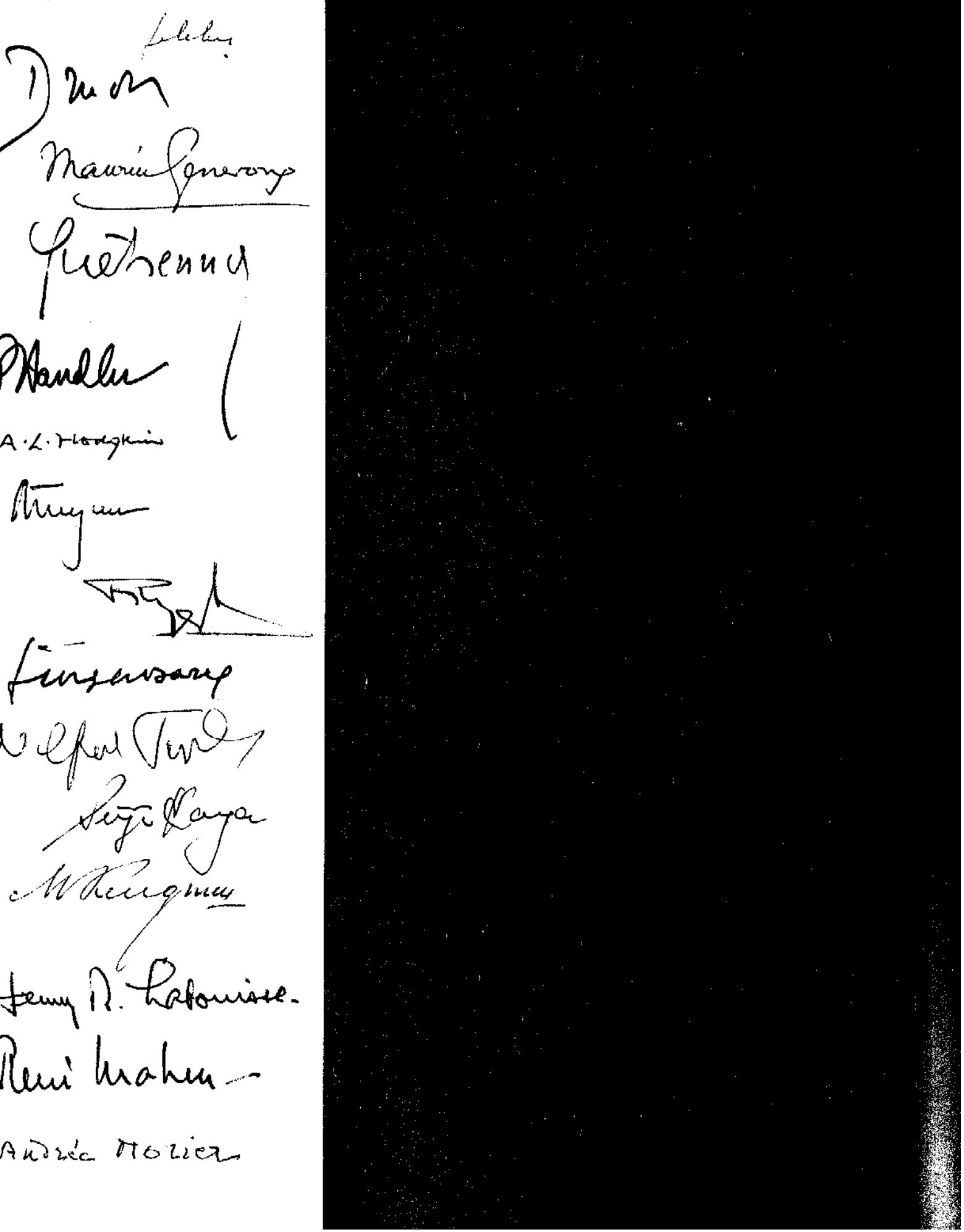


LES CAHIERS
DE
L'INSTITUT DE LA VIE

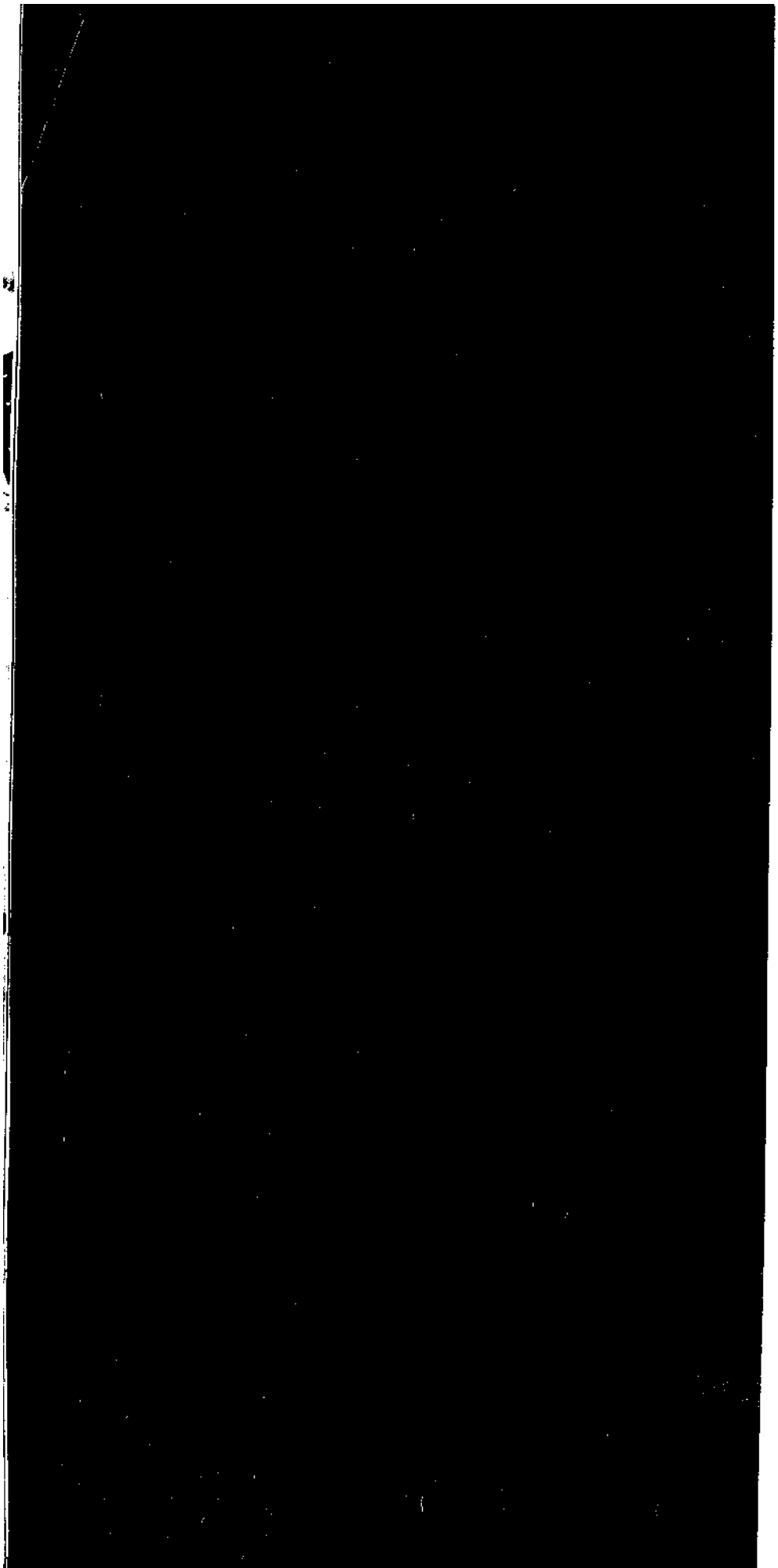
prix de l'institut de la vie

l'institut de la vie a créé en mille neuf cent soixante quatre un prix pour proposer à l'estime des hommes une personnalité ou une organisation qui aurait bien mérité de la vie soit pour l'avoir défendue, soit pour l'avoir illustrée. (cf. cahiers de l'institut de la vie, numéro deux, septembre mille neuf cent soixante quatre, page huit). à l'initiative de Jean Chenevier, président directeur général de la société française des pétroles BP et vice-président du conseil d'administration de l'institut de la vie, la société française des pétroles BP a décidé de doter ce prix à l'occasion de son cinquantenaire, en attribuant tous les deux ans une somme de deux cent cinquante mille francs. le prix s'intitule : « prix de l'institut de la vie » (fondation de la société française des pétroles BP pour l'institut de la vie). la société française des pétroles BP a entendu respecter la totale indépendance de l'institut de la vie et a souhaité laisser à l'institution l'entière responsabilité de déterminer la composition du jury. le conseil d'administration de l'institut de la vie a apprécié l'importance, la générosité et l'élégance de ce geste.

Jean Delay	de l'Académie Française
Maurice Druon	de l'Académie Française
Maurice Genevoix	Secrétaire Perpétuel de l'Académie Française
Jean Guehenno	de l'Académie Française
Philip Handler	Président de l'Académie Nationale des Sciences de Washington
* A. L. Hodgkin	President of the Royal Society
Rodolphe Hottinguer	Président de la Chambre Internationale de Commerce
René Huyghe	de l'Académie Française
Georges Izard	de l'Académie Française
Wilfrid Jenks	Directeur Général du Bureau International du Travail
Seiji Kaya	Membre de l'Académie des Sciences du Japon
* M. B. Keldych	Président de l'Académie des Sciences de l'U.R.S.S.
H. R. Labouisse	Directeur Général de l'U.N.I.C.E.F.
René Maheu	Directeur Général de l'U.N.E.S.C.O.
Andrée Morier	Président d'Honneur de l'Union Internationale de la Protection de l'Enfance



A. L. Hodgkin



Marcel Naville

Philip Noel-Baker

Wladimir d'Ormesson

L. B. Pearson

Manuel Perez-Guerero

Jean Rostand

Jacques Rueff

* Léopold Sedar Senghor

Pierre Henri Simon

* S. L. Sobolev

Jean Vanier

Etienne Wolff

SECRÉTAIRE PERPÉTUEL

Maurice Marois

Président du Comité International de la Croix Rouge

Prix Nobel de la Paix

de l'Académie Française

Prix Nobel de la Paix

Secrétaire Général de la Conférence des Nations Unies sur le Commerce et le Développement

de l'Académie Française

de l'Académie Française, Chancelier de l'Institut de France

Président de la République du Sénégal

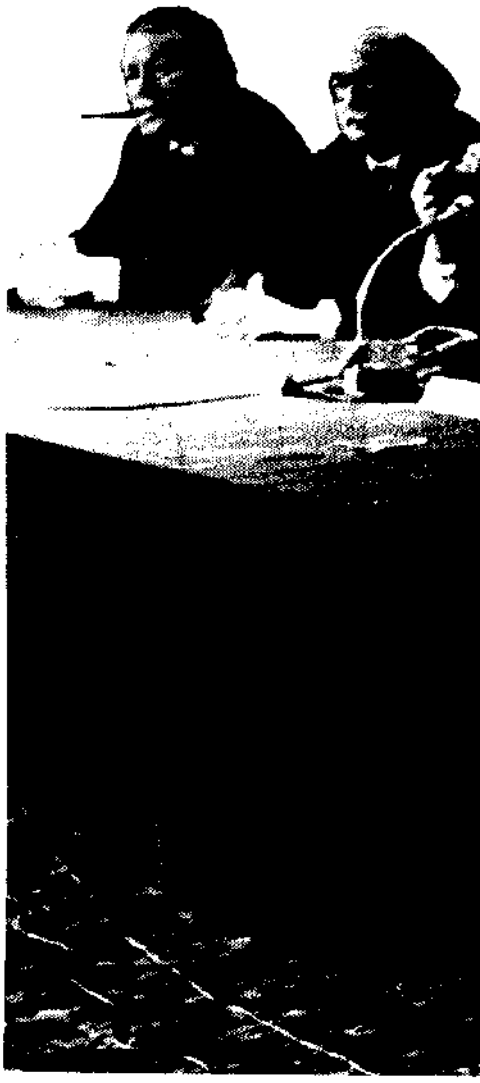
de l'Académie Française

de l'Académie des Sciences de l'U.R.S.S.

de l'Académie Française et de l'Académie des Sciences

Président du Conseil d'Administration de l'Institut de la Vie

le jury s'est réuni en séance plénière le deux juin mille neuf cent soixante et onze à Paris. la proclamation du lauréat a eu lieu immédiatement après le vote au cours d'une conférence de presse tenue dans les salons de l'union interalliée à Paris, en présence de monsieur René Cassin, président du jury de messieurs Louis Armand, Michel Cépède, Maurice Druon, Maurice Marois, Jean Rostand, Jacques Rueff, membres du jury et de monsieur Jean Chenevier, président directeur général de la société française des pétroles BP, vice président du conseil d'administration de l'institut de la vie.



De gauche à droite :
Maurice DRUON, Jean ROSTAND, Michel CEPEDE, René CASSIN, Maurice MAROIS, Jacques RUEFF, Louis ARMAND, Jean CHENEVIER.

extraits de la conférence de presse

RENÉ CASSIN

Le jury a examiné d'assez nombreuses candidatures, toutes dignes d'être présentées. Il a préféré couronner un homme qui n'était pas exclusivement de recherche, pour ne pas empiéter sur le prix Nobel; il a voulu mettre en lumière les mérites d'un praticien très expert, très compétent, qui a donné dans sa vie une application particulière des vœux des fondateurs du prix, à savoir : défendre et protéger la vie humaine; le jury a estimé bon de couronner un homme qui défend la vie à son origine, au moment où l'enfant n'est pas encore complètement formé; il a entendu couronner en même temps le courage civique du médecin praticien : celui-ci a constaté les méfaits d'un remède, il les a signalés et a ainsi sauvé la vie de quantité d'enfants. C'est donc à l'ensemble des médecins

praticiens qu'en la personne de Monsieur McBride le jury a voulu rendre hommage.

Nous avons fait notre devoir et maintenant nous allons demander si des personnes présentes ici et notamment des membres de la Presse veulent poser des questions.

QUESTION : COMMENT McBRIDE A-T-IL FAIT SA DÉCOUVERTE ?

JEAN ROSTAND

Je pense que ce n'est pas par des recherches expérimentales que M. McBride a montré l'action tératogène de la thalidomide, c'est par son observation clinique simplement, vigilante; le médecin allemand, Lentz, a également constaté une corrélation entre l'absorption de thalidomide et la production de bébés phocomèles; ce qui est important à dire c'est que le Docteur Lentz a lui-même reconnu la priorité de McBride et il l'a reconnue d'une façon très loyale, d'une loyauté à laquelle il faut rendre hommage.

MAURICE MAROIS

Je voudrais répondre à mon tour. Voici une publication de INGALLS, l'un des plus grands spécialistes d'obstétrique et de gynécologie des Etats-Unis, qui rend compte des circonstances de la découverte de McBride. L'histoire se passe en 1960. La thalidomide, qui s'appelait le distaval, avait été adressée en échantillon par le fabricant, la Distillers Company, à l'hôpital pour femmes de Sydney, en Australie, pour que soient entreprises des recherches cliniques sur son efficacité contre les vomissements du début de la grossesse. Le Docteur McBride a constaté, dans le service hospitalier qu'il dirigeait, trois cas d'absence congénitale de radius chez trois enfants qui sont nés dans une période de six mois après l'administration de

cette drogue. Ces trois enfants sont morts à la suite d'une atrésie du tube digestif, en dépit des tentatives opératoires pour essayer de les sauver. Les observations furent communiquées aux représentants de la Distillers Company en Grande-Bretagne par le Docteur McBride.

Le 14 juin 1961, l'hôpital pour femmes avait achevé ses travaux cliniques mais les vues du Docteur McBride n'avaient pas prévalu. Cependant, la confirmation est venue lorsque trois enfants malformés sont nés un peu plus tard, en été 1961.

Voilà donc rapportées par une haute autorité les circonstances de la découverte de McBride.

JEAN ROSTAND

Je veux simplement ajouter quelques mots. Ces observations ont été possibles parce que les biologistes savaient déjà par la voie expérimentale que des anomalies pouvaient être provoquées par des agents chimiques. Je crois qu'il est utile aujourd'hui de rappeler l'importance considérable de l'œuvre d'Ancel, le créateur de la chimiotératogenèse mais Ancel est malheureusement disparu. Si le Professeur Ancel vivait toujours, il recevrait certainement un des grands prix de l'Institut de la Vie car il a montré le premier avec sa fille, Madame Suzanne Lallemand, que des produits chimiques administrés au cours du développement embryonnaire pouvaient déterminer des malformations. Il faut saluer le nom du Professeur Ancel à cette occasion.

QUESTION : QU'EST-CE QUI A AMENÉ LA S.F. BP A S'INTÉRESSER A CETTE FONDATION ?

JEAN CHENEVIER

Je vais vous répondre en deux mots. Je suis membre de l'Institut de la Vie depuis pratiquement

son origine et, comme le Professeur Marois l'a rappelé tout à l'heure, j'en suis Vice-Président depuis quelques années. J'ai toujours pensé que les entreprises en général, et les plus grandes d'entre elles en particulier, avaient envers la société des responsabilités qui allaient bien au-delà de leurs responsabilités industrielles et commerciales; je crois que c'est une idée qui commence à se répandre dans tous les pays, et très spécialement aux Etats-Unis. C'est le sens de l'action que ma Société et moi-même nous essayons de mener dans différentes instances, en particulier au Centre des Recherches des Chefs d'Entreprises dont je suis Président, et cela explique également ma participation à l'Institut de la Vie. C'est vraiment la seule raison qui, au moment où notre Société célèbre cette année son cinquantenaire, nous a amenés à doter le prix de l'Institut de la Vie. Nous avons voulu faire cette fondation aussi désintéressée que possible. Nous n'avons pas créé de prix proprement nommé par notre Société. Personne de ma Société ne fait partie du jury.

JACQUES RUEFF

Je voudrais souligner la très haute portée intellectuelle, scientifique et sociale du geste que Monsieur Chenevier, au nom de la Société des Pétroles BP, nous permet d'accomplir aujourd'hui. Ce serait un lieu commun d'insister sur l'importance que joue le prix Nobel dans le progrès scientifique. Nous n'avions rien d'équivalent; dorénavant nous avons quelque chose qui est extrêmement proche du prix Nobel tout en s'en distinguant par le choix des thèmes. On peut aussi dire que dans les pays anglo-saxons, spécialement aux Etats-Unis, les Fondations ont joué un rôle immense dans le progrès des connaissances scientifiques et dans le développement de la culture.

Nous avons en France réalisé quelques progrès dans cette voie par la création récente de la Fondation de France; le geste généreux des Pétroles BP nous achemine dans la voie des fondations privées. Le jury est heureux de rendre hommage à la générosité et à la haute inspiration des fondateurs qui ont permis, pour la première fois aujourd'hui, de décerner le Prix de l'Institut de la Vie.

JEAN ROSTAND

Je voudrais simplement rappeler, tant pis si la modestie de Maurice Marois en souffre, que l'Institut de la Vie a été fondé il y a 11 ans et Maurice Marois m'en parlait il y a peut-être 13 ou 14 ans. Or maintenant, il n'est plus question que de défense de l'homme, de défense de la nature, de défense de la vie. C'est devenu une banalité, un lieu commun. Mais il y a 13 ou 14 ans, ce n'était pas un lieu commun, c'était une œuvre parfaitement originale, audacieuse et Maurice Marois s'est montré là un précurseur et un promoteur éminemment perspicace. Je tiens à le rappeler.

RENÉ CASSIN

Après avoir rendu hommage au Professeur Marois, je tiens à me joindre à ceux des membres du jury qui rappellent les mérites du donateur. Lorsque le Prix Nobel est décerné, les bénéficiaires font généralement l'éloge de Nobel décédé. Ici nous avons la joie de faire l'éloge d'un donateur bien vivant. Nous souhaitons qu'il fasse école et que longtemps ce prix soit décerné à des hommes le méritant vraiment pour que sa valeur non seulement pécuniaire mais morale soit conservée aux yeux de l'opinion publique.

Au premier rang :
Madame McBRIDE
et ses quatre enfants.

CÉRÉMONIE SOLENNELLE DE REMISE DU PRIX DE L'INSTITUT DE LA VIE DANS LA GALERIE DES GLACES DU CHATEAU DE VERSAILLES

22 JUIN 1971

ADRESS OF M. MAURICE MAROIS

The Institut de la Vie is a song to the glory of life.

" The wind rises, one must try to live ". So said Paul Valery. Since the arrival of man the wind has risen and they have tried to live. They have tried to live and they have lived, since we are here, living, the present link of a chain of generations, coming from the depth of bygone ages and which, link after link, should continue in the course of time.

Being shaped by the trends of thirty millions of centuries, the life has not been improvised. It has a policy : to persevere, and to express itself. It is destined to a brillant future.

A brillant future, indeed, but with or without mankind. Because life is threatened in its most elaborate forms. The ransom of organisation is greater weakness. At the summit of evolution, man holds the means to stop the course of its fate.

Mankind knows that it can die by a premeditated death. It is discovering that its ecological environment is frail and vulnerable and that it can be ruined in an irreversible way. It knows that its expansion will meet a limit because a day will come of the human saturation of the earth. Little by little it develops a conscience for the species; a conscience of a single community facing the same fate and same perils.

Man is now re-experiencing the panic and terror of earlier times, in face of the uncontrolled manifestations of the might of nature, being confronted with the forces he himself has released and with the acceleration of his own history. But, this feeling is modified at the same time by the realisation of being master of these unleashed forces, that is to say a free actor who controls and no longer a passive and powerless object of events. However, the realization of this apparent omnipotence of the creator brings in train the doubt for the well-ordered future : how to use the powers of science to the greater benefit of mankind. This is the dilemma : the exploitation of resources until they are exhausted, the extinction of the

ALLOCUTION DE M. MAURICE

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Modelée par l'effort de trente millions de siècles, la vie n'a pas été improvisée. Elle a une politique : persévérer, s'exprimer. Elle est appelée à un grand avenir.

Grand avenir, certes, mais avec ou sans l'homme. Car la vie est menacée dans ses formes supérieures. La rançon de l'organisation est une plus grande fragilité. Au sommet de l'évolution, l'homme détient aujourd'hui le moyen d'interrompre le fil de son destin.

L'humanité sait qu'elle est mortelle et d'une mort qu'elle peut se donner. Elle découvre que son milieu écologique est fragile et vulnérable et qu'elle peut l'altérer d'une manière irréversible. Elle sait que son expansion connaîtra sa limite car le jour viendra de la saturation humaine de la terre. Elle développe peu à peu sa conscience d'espèce : conscience d'appartenir à une même communauté soumise au même destin et aux mêmes périls.

La terreur panique des premiers âges face au déferlement incontrôlé des forces de la nature, voici que l'homme l'éprouve à nouveau devant la dimension des forces qu'il a lui-même libérées et l'accélération de sa propre histoire. Mais ce sentiment se tempère aussitôt, de la conscience d'être lui-même l'ordonnateur des forces déchainées, le sujet libre d'une histoire dominée et non plus l'objet impuissant et passif d'une histoire subie. Puis à l'ivresse de l'apparente toute puissance du démiurge fait suite l'inquiétude des lendemains lucides : comment utiliser les pouvoirs de la science pour un plus grand accomplissement de l'homme. Le voici devant l'alternative : exploitation des ressources jusqu'à épuisement, extinction des espèces, affrontement ultime entre

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MAROIS

le règne minéral et le règne vivant réduit à son suprême représentant, l'homme — ou : participation consciente, rationnelle, mesurée aux cycles biologiques, réconciliation de l'homme prométhéen et de la nature tutélaire, intégration dans un ordre cosmique où la vie apparaît comme une forme supérieure d'organisation, et la liberté comme la suprême acquisition de la vie.

Dans le tumulte de l'histoire contemporaine, on perçoit l'attente d'une nouvelle renaissance, d'un humanisme des temps nouveaux qui puisera sa lumière à deux sources : la reconnaissance de la valeur de la vie, l'affirmation de la grandeur de l'homme libre et responsable.

La nécessité s'impose d'édifier une structure d'accueil pour le monde qui vient, d'aller plus loin que l'histoire, de la gagner de vitesse pour que les valeurs éprouvées comme permanentes et universelles survivent à l'écroulement du monde périmé qui les a produites. La nécessité s'impose d'un organe d'espèce. Il intégrerait toutes les richesses, toutes les sagesses du passé. Il analyserait toutes les potentialités du futur et les confronterait aux aspirations et aux exigences permanentes de l'homme « indivis, temporel et intemporel » (Saint-John Perse).

Le 8 septembre 1960, nous avons invité les hommes de science à s'unir aux hommes du plus haut niveau de conscience, afin d'édifier une institution, l'Institut de la Vie, qui aurait valeur de symbole. Il faut que les hommes sachent qu'il existe quelque part sur la terre un haut lieu où l'humanité entreprend une méditation sur elle-même, sur son passé, sur son avenir, sur sa responsabilité envers la vie.

L'institution se développe puissamment sur tous les continents, portée par l'espérance des hommes et le mouvement de l'histoire.

L'Institut de la Vie est un acte de psychologie totale. Face à l'ivresse sanglante des paroxysmes où triomphe la mort, nous organisons un autre paroxysme, celui de la vie. Il ne s'agit nullement d'une exaltation collective mais d'une communion lucide, joyeuse et grave dans l'ardente affirmation de la volonté de vivre.

Chaque homme détient une parcelle de l'espérance de vie, une frêle étincelle. Toutes ces étincelles peuvent être rassemblées en un immense brasier.

species and the ultimate contest between the material forces and the living forces reduced to its supreme expression, man — or : a conscious and rational participation attuned to the rhythm of the biological cycle reconciling promethean man and his natural environment, integrating in a cosmic order where life appears as a superior form of organisation, and liberty as the highest acquisition of life.

Within the disorders of contemporary history one can recognise the expectancy of a new renaissance : emboding a new humanism which will draw its force from two sources — the recognition of the value of life; and the reassertion of the importance of a mankind free but responsible.

It has become necessary to construct a responsive foundation for future generations, to go further than history, in order to gain time so that the value which have proved themselves as permanent and universal survive the disintegration of a dissipated world has produced them. It has become necessary to create a framework for the species. It should integrate all the richness and wisdom of the past. It should analyse all the potentials of the future and confront them with the hopes and continual demands of man " undefined, temporal and spiritual ". (Saint-John Perse).

On the 8 September 1960, we we invited scientists to meet with men of the highest level of thought, in order to create an institution, l'Institut de la Vie, which would act as a symbol. It is necessary that men realise that there exists somewhere on earth a high place where men undertake to reflect upon humanity, its past and future, and its responsabilities towards life.

The institution is developing strongly throughout the world, carried by the hope of men and the tide of history.

L'Institut de la Vie is a response to psychological needs. Faced with the frenzied and cruel paroxisms where death triumphs, we are promoting another paroxism, that of life. It is in no way a collective exaltation but an act of communion, calm, joyful and solemn, in the firm belief of the will to live. Each man holds a fragment of the

L'Institut de la Vie est un projet moral et politique. Il propose une vision globalisante. Il veut aider les hommes dans leur marche vers l'unité, unité de la connaissance, unité du genre humain. Il croit à la puissance d'appel des symboles et c'est pourquoi il a créé le Prix de l'Institut de la Vie afin de proposer à l'estime des hommes une personne qui a bien mérité de la vie soit pour l'avoir défendue, soit pour l'avoir illustrée.

Ce Prix vient d'être doté de 25 millions d'anciens francs tous les deux ans, à l'initiative de Jean Chénevier, l'un des vice-présidents de l'Institut de la Vie, par la Société Française des Pétroles BP, à l'occasion de son cinquantenaire.

Dans le jury siègent côte à côte les Présidents, Directeurs Généraux ou Secrétaires Généraux d'institutions mondiales qui ont pour ultime objet l'homme. Elles veillent sur son enfance, s'inquiètent de sa santé, de son labeur, des conditions de son développement. Elles pourvoient à ses nourritures terrestres et spirituelles; elles incarnent le grand rêve d'unité puisque c'est à l'échelle de la planète qu'elles accomplissent leur mission. D'autres attestent la fraternité au cœur même de la mêlée des guerres, d'autres, la coopération; d'autres se vouent aux échanges internationaux : elles tissent la trame d'un monde qui pourrait être solidaire. Aux représentants au sommet de ces grandes institutions se joignent des témoins : témoins de la civilisation de l'universel; témoins exemplaires du combat pour la paix mené par des hommes pleinièrement voués, avec toutes les ressources de leur être à la passion de la paix; témoins de l'attention à la vie et à l'homme au nom de l'amour; témoins de la plus haute science.

Mesurez l'autorité morale de ce jury mondial, bouquet de symboles, signe des temps d'une humanité qui puise en elle-même la volonté de vivre en mobilisant dans un élan unifié ses forces vives.

Cette fête des symboles est plus éclatante encore par les circonstances de la remise solennelle du Prix.

Cette cérémonie a lieu en ce 22 juin 1971, au cours du Congrès de Physique Théorique et de Biologie. Tous les deux ans, depuis 1967, l'Institut de la Vie réunit l'expression suprême du génie scientifique, cent des plus grands savants de la terre;

hope of life, no more than a tiny spark. But all these sparks could be put together to form an immense fire.

L'Institut de la Vie is a moral and political undertaking. It proposes a universal vision. It can help men in their progress towards a unity, a unity both of knowledge and of the human environment. It believes in the power of symbolic gestures and it is why the Institut de la Vie as a token of mankind's esteem to a person for services rendered in the defense or enrichment of life.

This prize is now endowed with the sum of 25,000,000 old francs every other year, thanks to the initiative of Jean Chenevier, one of the Vice-Presidents of the Institut de la Vie, by la Société Française des Pétroles BP, on the occasion of its half centenary.

The jury comprises of Presidents, Chaimen and Directors of international institutions which have one object in common — man. They survey his upbringing, examine his health, his working conditions and his development. They keep wath over his earthly and spiritual needs; they embody the great dream of unity since they operate throughout the world. Some give evidence of fraternity even in the midst of wars; some work towards international exchanges and co-operation; they weave the fabric of the world as though is were united. Representatives of these outstanding Institutions are joined by witnesses : witnesses of the universal civilization; witnesses of the fight for peace led by devout men giving fully of themselves for peace; witnesses of the attention to life and to mankind in the name of love; witnesses of the highest learning.

Consider the emminence of this international jury, a symbolic synthesis, a sign of the times in which mankind manifests its will for life by embodying in such a unified gesture its living forces.

This symbolic event is all the more outstanding in view of the circumstances of the ceremonial presentation of the prize. This ceremony takes place on this 22nd day of June 1971, in conjunction with the Congress of Theoretical Physics and Biology. Every two years, since 1967 the Institut de la Vie reunites the

il leur pose la question éternelle : « Qu'est-ce que la vie ? » et leur demande de répondre avec les ressources de la science contemporaine.

Rassemblés en un seul corps, ces savants interrogent l'univers où nous sommes plongés et la vie qui nous anime. Ils scrutent les conséquences de leurs découvertes pour la vie de l'homme et des sociétés; ils décident de poursuivre leur œuvre d'exploration, de quête incessante qui est l'honneur de l'homme; mais ils mesurent aussi leur devoir d'information envers la communauté des hommes.

La coïncidence des deux manifestations est voulue. Ainsi est établi un lien visible entre la plus profonde réflexion scientifique sur la vie et la reconnaissance de sa valeur.

Je rends un respectueux hommage à Monsieur le Président de la République Française qui a fait à l'Institut de la Vie le grand honneur d'accorder son haut patronage à la conférence et de désigner, pour le représenter, Monsieur le Ministre de l'Environnement — l'environnement, thème de vie. Pour la première fois, un chef d'Etat accorde son haut patronage à une manifestation de l'Institut de la Vie : ce geste a valeur de signe.

Cette fête des symboles n'est pas une incantation pour conjurer le désespoir de l'espèce moribonde à la dernière station du chemin d'angoisse. Nous ne créons pas un mythe démobilisateur, nous ne nous berçons pas dans l'illusion lyrique mais dérisoire. Nous ne sommes pas faits de l'étoffe des rêves. Il ne s'agit pas d'un superbe jeu de scène. Cet événement est un avènement. L'utopie qui nourrit notre espérance est une utopie motrice; elle sera la réalité de demain. Elle est portée par une force vieille de 30 millions de siècles : la vie. Elle est sensible à l'appel des millénaires futurs dont elle veut transformer l'incertitude en promesse. Elle est sous-tendue par notre volonté organisatrice.

Avec une intensité aigüe, je vis cet instant privilégié, cet instant unique où la conscience collective perçoit enfin la vivante unité du groupe humain.

L'événement est irréversible : nous fêtons aujourd'hui la réconciliation de l'homme avec lui-même. Nous scellons l'alliance de l'homme et de la vie.

highest expression of scientific genius, a hundred of the worlds most learned men, and asks them the eternal question " what is life " and ask them to reply with their resources of contemporary knowledge.

Assembled in a single group these men pose questions about the universe in which we find ourselves and the life therein. They examine the consequences of their discoveries for the life of mankind and its societies; they decide to pursue their works of exploration, the incessant searching which is the hallmark of man; but they appreciate also their duty to inform the community of men.

The co-incidence of these two meetings is deliberate. It is done to establish a visible link between the profound scientific thoughts on life and the recognition of its value.

I would like to pay homage to the President of the French Republic who has accorded to the Institut de la Vie the great honour of his esteemed patronage to the Conference and who has designated, to represent him, the Minister of Environment — the environment which is the theme of life. For the first time a Head of State gives his distinguished patronage to a meeting of the Institut de la Vie; this gesture has a value of significance.

This symbolic occasion is not an incantation to conjure up the despair of a declining species in its last stages of anguish. We do not live in the world of illusions. We are not made of the stuff of dreams. It is not a question of a showpiece. This event is an advent. The utopia which nourishes our hopes is a tangible utopia; it will be the reality of tomorrow. It is carried forward by a force sustained over 30 millions centuries : the life. It is responsible to the call of future the uncertainty of which has to be transformed into a promise. It is sustained by our conscious will.

With a profound intensity I cherish this priviledged moment, a unique moment when the collective conscience recognises at last the living unity of mankind.

The event is irreversible; we celebrate today the reconciliation of man with himself. We seal the pact between mankind and life.

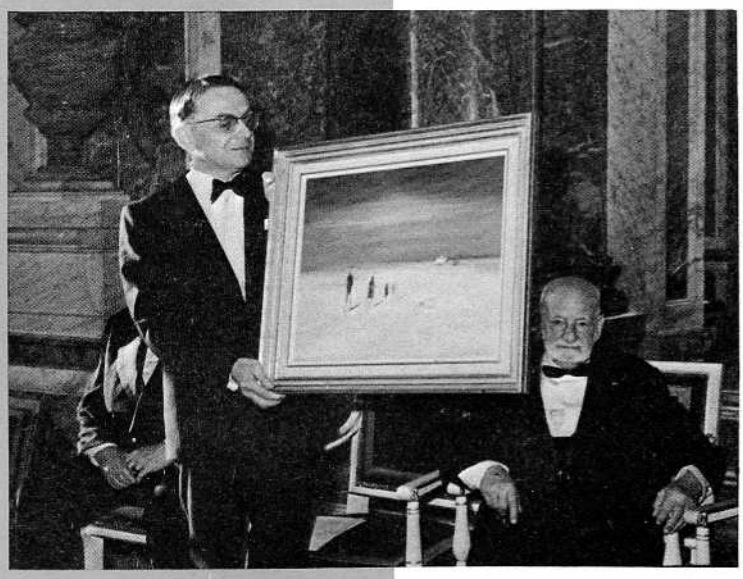

Tableau offert par
William McBRIDE

ALLOCUTI

De gauche à droite :
René CASSIN,
William McBRIDE,
Jean CHENEVIER.

N DE M. RENÉ CASSIN

Monsieur le Ministre, Monsieur le Président, Mesdames, Messieurs,

C'est pour moi un grand honneur que d'avoir été chargé de remettre à Monsieur le Docteur McBride, le premier des prix décernés par l'Institut de la Vie, grâce à l'initiative généreuse de Monsieur Chénevier.

Depuis des millénaires, le Décalogue a formulé pour tous les hommes, le précepte négatif « tu ne tueras point » qui hélas ! est souvent violé. Mais de tout temps aussi une catégorie particulière d'hommes, les médecins, a été assujettie à une mission positive. « Tu conserveras et défendras la vie ». Monsieur McBride est un de ceux qui donnent l'exemple dans l'accomplissement de cette mission. Il illustre en même temps le principe que la science est au service de l'homme et cesserait de mériter sa place s'il en était autrement.

Or, dans le monde moderne, son rôle est devenu si considérable qu'il est nécessaire qu'un nombre croissant de personnes prenne conscience du droit à l'existence que possède chaque être humain et du devoir pour l'ensemble des autres de respecter sa vie.

Depuis un demi-siècle de très nombreux hommes ont eu la terrible obligation de participer à deux guerres meurtrières et d'exposer leur propre vie pour faire obstacle au triomphe de la violence et de la barbarie. J'ai été de ceux-là. Aussi ma fierté est-elle d'autant plus grande d'exalter et honorer ceux des hommes qui, comme le Docteur McBride, ont le sens de la solidarité humaine et pratiquent celle-ci avec science et dévouement.

Qu'il me soit permis au moment où je vais procéder à la remise au lauréat du prix décerné par l'Institut de la Vie, de lire la décision motivée du Jury.

« Le jury du Prix de l'Institut de la Vie a attribué pour la première fois le Prix de l'Institut de la Vie (Fondation de la Société Française des Pétroles BP pour l'Institut de la Vie) d'un montant de 250 000 francs (25 millions da'nciens francs) à Monsieur le Docteur William McBride, gynécologue accoucheur de l'Université de Sydney (Australie) âgé de 44 ans. McBride a incontestablement, le premier, dénoncé un cas d'agression flagrante contre la vie : l'action tératogène de la thalidomide sur l'embryon humain. Médecin praticien, il incarne la vigilance thérapeutique, le souci de l'avènement d'un être sain à la vie. Le service ainsi rendu à l'humanité est exemplaire. En retenant son nom, c'est à l'ensemble des médecins praticiens du monde que le jury rend hommage ».

Visiter cette grande ville, apprécier l'élégance et la beauté de ses bâtiments et tout particulièrement de ce magnifique palais et de ses jardins, me rappelle que nous sommes les usufruitiers de ce monde; en tant que tels, nous ne devons pas altérer notre environnement ni piller nos ressources naturelles, mais au contraire nous efforcer de laisser notre monde en meilleur état que nous ne l'avons trouvé. Quelle plus grande cause pouvons nous servir que d'alléger le poids de la misère humaine.

Quel que soit le système politique qui nous régit ou l'environnement géographique dans lequel nous vivons, le devoir commun de tout homme est de veiller à ce que chaque enfant qui doit naître naisse en bonne santé, libre de tout défaut de développement, qu'il ne soit pas handicapé par un retard mental et qu'il ne meure pas de maladie infectieuse ou de faim.

Sans aucun doute, les quarante dernières années ont vu se réaliser de grands progrès. La découverte de la chimiothérapie et des antibiotiques, ainsi que le contrôle de la malaria ont considérablement abaissé le taux de mortalité, ce qui constitue l'un des plus admirables progrès. Mais ce progrès a pour effet secondaire le doublement de la population du globe en trente ans. Si la tendance actuelle persiste, à la fin de ce siècle, quatre cinquièmes de la population du monde vivront en Asie, en Afrique ou en Amérique Latine. Le taux de famine sera dans trente ans le double de ce qu'il est aujourd'hui. Les problèmes de surpopulation et de malnutrition sont urgents et impératifs : ils exigent beaucoup plus d'attention que nous ne leur en accordons.

La production massive de moteurs à combustion interne, le développement des matières plastiques, des détergents et pesticides chimiques ont créé des

ALLOCUTIODE

To visit this great city, to ponder on the elegance and beauty of the buildings and especially this magnificent palace and gardens, serves to remind me that we are the tenants of this world and as such should not despoil our environment or plunder all our natural ressources, we must aim to leave our world better than we found it.

What greater challenge can we be given that to lighten the burden of human misery.

The common concern of man, no matter what Political System we follow or what the geographical environment in which we live, is to see that each child born, will be born healthy, free from developmental defects, not impaired by mental retardation and not die from infection or starvation.

Undoubtedly, the last forty years have seen great advances. The discovery of chemotherapy and antibiotics, and the control of malaria have drastically reduced the death rate which is one of the most wonderful of all advances. However, this has produced the side effect that the worlds population will double in 30 years. If the present trend continues, by the turn of the century, four fifths of the worlds population will live in Asia, Africa or Latin America. The hunger rate in thirty years time will be double what it is today. The problems of overpopulation and malnutrition are urgent and pressing ones demanding much more attention than we are devoting to them.

The mass production of the internal combustion engine, the development of plastics, detergents, and chemical pesticides have

DE W. G. McBRIDE

given us environmental pollution problems undreamed of, even by the most advanced thinkers thirty years ago.

The carbon monoxide in the air, mercury in the sea water, radio activity in the atmosphere, give us problems which we must solve and solve quickly if we are to leave a heritage for our children.

Not only must we think of pollution of the external environment but also pollution of the internal environment. The great significance of the Thalidomide tragedy is to show us just how vulnerable the fœtus is to its environment. The fact that ten years after the event we are still unsure of the mechanism of its effects on the developing tissues surely emphasises that we must make much more effort and spend much more money on the study of life.

Is there need for haste in exploration of space until we have made this planet free from overpopulation, poverty, starvation and disease. Space will be conquered but could it not be done as a co-operative project at leisure without undue haste ?

Man has produced magnificent scientific aids. Let them be used for the benefit and not the destruction of the human race. Contented people do not become aggressors. One has only to read the history of the past fifty years to see the futility of war.

On seeing this great gathering of learned men and women from so many countries of the world, all dedicated to the same objective, it gives me great confidence that mankind has the capacity to cope with the new problems which arise.

problèmes de pollution de l'environnement que les penseurs les plus hardis ne pouvaient imaginer il y a trente ans.

L'oxyde de carbone dans l'air, le mercure dans l'eau de mer, la radioactivité dans l'atmosphère nous posent des problèmes que nous devons résoudre et résoudre rapidement si nous voulons laisser un héritage à nos enfants.

Nous ne devons pas penser seulement à la pollution de l'environnement extérieur mais aussi à celle de l'environnement interne. Le grand enseignement de la tragédie de la Thalidomide est justement de nous montrer à quel point le fœtus est sensible à son environnement. Le fait que dix ans après cet événement nous ne connaissons toujours pas d'une manière certaine le mécanisme de ses effets sur les tissus en développement, souligne avec éclat la nécessité où nous nous trouvons de consacrer beaucoup plus d'efforts et beaucoup plus d'argent à l'étude de la vie.

Est-il besoin de se hâter d'explorer l'espace avant d'avoir libéré notre planète de la surpopulation, de la misère, de la famine et de la maladie. L'espace devra être conquis mais cette conquête pourrait-elle se réaliser en coopération, à loisir, sans hâte inutile ?

L'homme s'est magnifiquement donné les secours de la science. Utilisons-les pour le bénéfice et non pour la destruction du genre humain. Les peuples satisfaits ne deviennent pas des agresseurs. Il n'est que de lire l'histoire des cinquante dernières années pour prendre conscience de la vanité des guerres.

Le spectacle de cette importante réunion d'hommes et de femmes de haute pensée, de tant de pays du monde, tous dévoués à la même cause nous donne confiance : l'humanité est capable de faire face aux problèmes nouveaux qui surgissent sur son chemin.

ALLOCUTION DE M. ROBERT POUJADE

Messieurs les Présidents,
Monsieur le Ministre,
Messieurs les Membres des Académies et des Instituts,
Monsieur le Professeur,
Mesdames et Messieurs,

Ayant l'honneur de représenter Monsieur le Président de la République au Congrès de la Physique Théorique à la Biologie organisé par l'Institut de la Vie, je me trouve avoir aussi le plaisir d'assister à la remise solennelle de ce prix à Monsieur William McBride.

Monsieur le Président Marois, permettez-moi de vous dire que nul n'a pu être insensible à la ferveur avec laquelle vous venez de célébrer la vie, ferveur lyrique permettez-moi de le dire malgré vos dénégations, et vous me le permettrez je crois, d'autant plus volontiers que chacun sait que vous êtes à l'origine de cette manifestation, de ce rassemblement de tant de Savants mondialement connus et que votre ferveur est une ferveur active.

Vous êtes aussi à l'origine avec l'Institut de la Vie, de cette réunion de ce soir dans un cadre prestigieux dont le but est d'honorer un chercheur qui a défendu la Vie dans ses principes et dans ses origines.

Je voudrais, Monsieur le Professeur, être en mesure de dire avec plus de compétence vos mérites. Le choix de l'Institut me dispense de rien y ajouter que les félicitations de Monsieur le Président de la République, auxquelles je me permets d'ajouter mes félicitations personnelles. Je dirai seulement que si j'avais dû décerner ce prix en fonction de ce que vous avez su dire en faveur de l'environnement, je vous l'aurais sûrement accordé, Monsieur le Professeur, avec plus de compétence.

En acceptant de patronner votre Congrès, Monsieur le Président de la République a témoigné de son souci de voir notre pays s'associer à toute entreprise qui s'efforce de dominer les problèmes de notre temps et de jeter les fondements d'un humanisme universel.

Hommes de Sciences, vous savez Messieurs, que toute recherche, tout humanisme, est tributaire d'un passé culturel, mais peut-être avons-nous besoin aujourd'hui, comme le disait naguère le Président Georges Pompidou, d'une nouvelle Renaissance. Ce qui a marqué la Renaissance, c'est un respect profond pour les valeurs du passé et c'est une quête ardente de valeurs nouvelles.

Ce respect et cette ardeur sont indissociables lorsqu'on ne veut être prisonnier, ni des habitudes du temps jadis, ni des modes du temps présent.

La curiosité de l'homme pour la Vie est bien sûr une curiosité à l'égard de soi-même.

Nous ne pouvons oublier ce qu'a de pathétique l'interrogation toujours sans réponse de la seule créature qui réfléchit à son destin. Mais le spectacle de la vie, est en soi-même si fascinant que beaucoup d'hommes, et en particulier d'hommes de Sciences

De gauche à droite :
François de CLERMONT-TONNERRE,
Maurice MAROIS, René CASSIN, Pierre POUJADE,
William McBRIDE, Jean CHENEVIER.

ont mis dans cette recherche plus d'enthousiasme que d'angoisse, même s'ils n'ont pas trouvé dans la foi, ce qu'ils ne trouvaient pas dans la connaissance. L'homme a frayé sa voie dans la nature, à travers la nature. Il a longuement confronté sa vie précaire à la vie puissante qui s'épanouissait dans la biosphère.

En face de la nature, il éprouvait un sentiment de frustration et parfois de révolte. Ce sentiment traverse toute notre littérature qui a opposé volontiers la fragilité de l'existence humaine, à la pérennité de la nature.

« Vivez, froide nature et revivez sans cesse » écrivait VIGNY au moment même où il reconnaissait dans l'apparition du chemin de fer une innovation qui pouvait changer la Vie.

Aujourd'hui, c'est la précarité de la vie dans la biosphère, qui tourmente l'homme contemporain. L'homme avance, et, comme nous le rappelait, il y a un instant, Monsieur le Professeur MCBRIDE, la nature recule.

Après avoir appris que les civilisations étaient mortelles, nous nous apercevons aujourd'hui, que c'est la Civilisation qui peut être mortelle. Il peut y avoir une terre sans soleil et il peut y avoir une terre sans vie ! Or l'homme est devenu un consommateur insatiable de la vie qui l'entoure, au moment où beaucoup d'espèces s'affaiblissent ou disparaissent. Certes, il se multiplie, mais pourra-t-il maintenir une vie digne d'être vécue dans une nature chaque jour diminuée ?

Bien sûr, dans la frénésie de création, qui l'a saisi depuis cent ans, et qui a apporté tant de progrès à la science, et convenons-en, Messieurs, tant de progrès en particulier à la Médecine, donc, tant d'apports à la vie, l'homme crée aussi de la vie. A des appétits nouveaux, il offre des nourritures nouvelles, à des exigences chaque jour renouvelées, il propose des techniques nouvelles de production et de productivité.

L'impression qui prédomine pourtant, est que notre espèce dans son appétit de vivre attaque les racines mêmes de la vie. La sagesse ancestrale, qui enseignait les équilibres du milieu naturel, disparaît. Aujourd'hui, seuls les écologistes détiennent des vérités que la sagesse des nations avait apprises tout au long de l'histoire et même, de la préhistoire.

Vous vous êtes interrogés, Messieurs, sur la physique théorique et sur la biologie, et je n'ai pas compétence pour vous suivre jusque dans ces abîmes de sciences, mais comment fait-on de la vie avec la mort ? Diderot nous a donné, dans l'entretien avec d'Alembert, la recette qui permet de passer d'une statue de Falconet à des pois, des fèves et des choux. Mais on connait aussi, et elle est d'une application plus facile et plus rapide, la recette qui permet de passer de la forêt au désert, et le désert est plus tenace que la forêt.

L'homme peut vivre, sans doute, en s'éloignant de la vie, et je dirais que l'expérience peut être passionnante; aujourd'hui, la rencontre entre la vie et la mort, s'accomplit dans le cosmos, grâce au génie humain !

Devons-nous nous préparer un avenir de cosmonautes?

Nous devons, je crois, nous souvenir que nous sommes des hommes de ce monde. Nous pourrons peut-être, pour combien de temps, dans quelles conditions, vivre d'une vie qui sera de plus en plus, ou qui risque d'être de plus en plus, la négation de ce que des générations ont appelé la vie.

C'est, je crois le problème de l'environnement.

On dit parfois qu'il met, ou qu'il remet tout en cause, et c'est vrai, précisément parce qu'il met en cause, la vie !

C'est pourquoi, Messieurs, le Professeur MAROIS a voulu associer, je pense à vos recherches, mes préoccupations et mes fonctions. Je n'exclus aucune hypothèse; on peut imaginer, on doit imaginer sans doute, une existence, une civilisation, un cadre de vie, aujourd'hui, presque inimaginables.

L'initiative humaine peut nous réserver de fantastiques surprises. Il est possible que, dans quelques siècles, on ne puisse distinguer ce que l'on appelle aujourd'hui naturel, et ce que l'on appelle artificiel; et dès aujourd'hui, cela est vrai dans quelques domaines. Quelques uns des grands lacs français sont des créations de l'homme, nous savons transformer les déchets de la société industrielle en forêt; nous essayons de plier l'arbre aux exigences du béton pour permettre leur coexistence.

On peut imaginer que l'homme mette les moyens de sa technique au service du remodelage d'un environnement qui échapperait à certaines lois actuelles du milieu naturel. Rien n'interdit de rêver, et même de rêver à un homme qui changerait de dimensions, sinon de nature, maîtrisant et dominant son environnement; et, s'il est permis de rêver, il est dans l'immédiat nécessaire d'imaginer, l'esprit attentif aux promesses du futur, mais aussi, aux nécessités d'aujourd'hui, un cadre de vie qui, sans métamorphose fondamentale et sans miracle, permette à l'homme de retrouver un équilibre dont chacun sait bien, qu'il est actuellement menacé sur le plan physique et sur le plan psychique.

Nous le recherchons et nous le recherchons avec beaucoup de peine, ce cadre qui donnerait la primauté à la qualité de la vie.

Force nous est bien de constater qu'en 1971, l'homme est obligé de maintenir son vieux pacte avec la terre, sa vieille entente avec la biosphère, et c'est pourquoi nous avons, Monsieur le Professeur, grand besoin d'hommes comme vous.

Si l'homme continue à menacer la vie autour de lui, c'est sa propre vie, c'est sa vie d'espèce qui sera menacée, car les biens essentiels, dont parlait à Chicago, le Président de la République, qui m'a fait le grand honneur de me déléguer auprès du congrès, demeurent irremplaçables : la terre, l'eau, l'air, les forêts.

Je crois traduire sa pensée, en vous disant, Monsieur le Professeur, Messieurs, vous tous qui avez illustré la science et la recherche, que nous souhaitons voir les plus grands esprits de la science moderne, donner un peu de leur temps et de leur génie, à ce problème de vie et de mort qu'est la sauvegarde de l'environnement humain.

INSTITUT DE LA VIE

PRIX DE L'INSTITUT DE LA VIE

(Fondation de la Société Française des Pétroles BP pour l'Institut de la Vie)

Le Jury International, réuni le 2 juin 1971,
sous la Présidence de Monsieur René Cassin,
Prix Nobel de la Paix,
a décerné ce prix à

Monsieur le Docteur William G. Mc Bride,
gynécologue obstétricien de l'Université de Sydney.

"Il a incontestablement le premier,
dénoncé un cas d'agression flagrante contre la vie :
l'action tératogénique de la thalidomide sur l'embryon humain.
Médecin praticien, il incarne la vigilance thérapeutique,
le souci de l'avènement d'un être sain à la vie.

Le service ainsi rendu à l'humanité est exemplaire.
En retenant son nom, c'est à l'ensemble
des médecins praticiens du monde
que le Jury rend hommage".

Versailles le 22 Juin 1971

le Président du Jury
R. CASSIN

le Secrétaire perpétuel du Jury
M. MAROIS

FONDATION
DE L'INSTITUT DE LA VIE
POUR L'ÉTUDE
DES SEMAINES
DE FORMATION
DE LA VIE HUMAINE

INITIATIVE DU Dr WILLIAM G. McBRIDE

Premier lauréat du Prix de l'Institut de la Vie
de créer une fondation de l'Institut de la Vie
pour la prévention des handicaps physiques et mentaux
Le 30 juin 1971

Paris, le 30 juin 1971

Monsieur le Président René CASSIN
Prix Nobel de la Paix
Président du Jury International
du Prix de l'Institut de la Vie
36, quai de Béthune, 75 - Paris (3e)

Dear President Cassin,

I wish to thank the Institut de la Vie for the great honour not only to me but also to my country Australia in my being awarded the Prix de l'Institut de la Vie.

I wish to inform you Sir, so that you may convey my intention to your committee that I intend to devote my prize money of 250 000 F to set up an Institut de la Vie World Foundation for the Study of the Formative Weeks of Human Life.

I do this, as I feel that the only way we can prevent physical malformation of the fœtus and mental retardation of children, is to make an intensive world wide study of these problems which plague every country no matter what political philosophy we follow or what the geographical environment in which we live.

To you Sir I regard it my great honour to have met you and made your acquaintance.

Yours faithfully
William G. McBRIDE

Paris (5e), le 2 juillet 1971

Monsieur le Docteur William G. McBRIDE
" Harley "
600 Railway Parade
Hurstville 2220 (Australie)

Cher Docteur McBride,

L'Institut de la Vie, son Président le Professeur Marois et le Jury du Prix qui vous a été décerné, ont reçu votre lettre de remerciement avec satisfaction.

Permettez-moi, à mon tour, de vous exprimer notre reconnaissance pour la généreuse décision que vous nous confirmez, de consacrer le montant de ce prix à une fondation dont vous avez fixé le nom, en omettant même de mentionner le vôtre.

Ainsi, vous tenez à mettre non seulement votre science, mais aussi les ressources dont vous disposez au service de la vie humaine et particulièrement de l'être humain dès avant sa naissance. Et vous tenez à le faire avec une modestie qui vous honore.

Soyez assuré que l'Institut de la Vie veillera à ce que votre but soit poursuivi. Il veillera en particulier à donner à votre fondation une solide situation juridique.

Permettez-moi d'ajouter à ces remerciements officiels l'expression du plaisir que j'ai trouvé à faire votre connaissance personnelle et celle de votre famille.

Veuillez agréer, je vous prie, cher Docteur McBride, l'expression renouvelée de ma très haute estime et de mes sentiments dévoués.

René CASSIN

PREMIÈRE CONFÉRENCE INTERNATIONALE

en vue de l'établissement
de la Fondation de l'Institut de la Vie
pour la prévention des handicapés physiques et mentaux

tenue au Centre de recherches,
d'étude et de formation mutualistes et sociales
de la Mutuelle générale de l'éducation nationale
du 3 *au* 5 *décembre* 1971

sous le patronage de

Monsieur Robert **BOULIN**
Ministre de la Santé publique et de la Sécurité sociale

et de

Mademoiselle Marie Madeleine **DIENESCH**
Secrétaire d'Etat

ALLOCUTIONS OFFICIELLES
OFFICIAL ADRESSES

Maurice MAROIS

Jean CHENEVIER

Son Excellence Monsieur l'Ambassadeur RENOUF
Ambassateur d'Australie en France

Message de Monsieur le Secrétaire Général des Nations Unies

ALLOCUTIONS D'OUVERTURE DE LA CONFÉRENCE

Maurice MAROIS

Robert DEBRÉ
Membre de l'Académie des Sciences

ALLOCUTIONS OFFICIELLES
OFFICIAL ADRESSES

M. Maurice MAROIS

Président du Conseil d'Administration de l'Institut de la Vie

Le 2 juin 1971, le jury international du Prix de l'Institut de la Vie, au terme de ses délibérations, a distingué le Docteur William McBride.

Or voici que le Docteur McBride consacre son prix à la création d'un fonds international pour la prévention des handicaps physiques et mentaux et lance un appel dans son pays pour augmenter la première dotation.

L'Institut de la Vie décide de l'aider en organisant la présente conférence.

Ici, la science fait son œuvre au service des hommes ; la médecine fait son œuvre dans une voie qui n'est pas seulement de guérir mais de prévenir ; l'Institut de la Vie fait son œuvre pour l'épanouissement d'une vie saine afin que l'homme soit l'homme.

L'Institut de la Vie fait son œuvre en rassemblant la science de toute la terre dans une recherche pluri-disciplinaire pour un combat d'une importance majeure : vaincre ce que le Professeur Turpin appelle le fléau congénital.

L'Institut de la Vie fait son œuvre en rassemblant les organisations et les institutions nationales et internationales à vocation scientifique, médicale, sociale, humanitaire. Ainsi, à ceux qui savent ce qu'est un handicapé, à ceux qui en portent la charge matérielle et morale, à ceux qui éprouvent le poids de la souffrance humaine, nous pouvons dire que la science et la médecine ne sont pas sans recours. Avec la prudence qui convient à la science, nous pouvons affirmer que les possibilités sont grandes et qu'il n'est pas déraisonnable de mobiliser des moyens puissants.

Une institution de pensée, d'espérance et d'action : l'Institut de la Vie; un jury international d'une immense valeur symbolique : le jury du Prix de l'Institut de la Vie ; un lauréat homme de lumière et figure de proue : William

McBride ; un thème qui concerne 10 % de la jeune humanité : les handicaps physiques et mentaux ; un appel à quelques-uns des plus grands noms de la science et de la médecine, suivi d'une réponse unanime ; une émulation de générosité entre Denis Forestier et la M.G.E.N., Jean Chenevier et la Société Française des Pétroles B.P., Charles Mérieux et l'Institut Mérieux ; la définition avec une rigueur extrême d'une stratégie scientifique et institutionnelle pour l'étude des semaines de formation de l'être humain, voilà l'événement qui rassemble en cette terre de France des hommes venus des points les plus éloignés du globe : d'Australie, de Belgique, du Canada, d'Espagne, des Etats-Unis, de Grande-Bretagne, d'Italie, du Japon, de l'Ouganda, de la République Fédérale d'Allemagne, de Suède.

Un à un, la science réalise les plus vieux rêves des hommes. Pourra-t-elle une fois encore conjurer la fatalité et donner un autre visage à la condition humaine ? La biologie dans un avenir prévisible rendra-t-elle accessible le rêve des rêves : tenter de faire mieux que la nature en modelant l'homme à notre gré ? Modifierons-nous radicalement notre propre définition ? Jusqu'où irons-nous trop loin ? Y a-t-il un trop loin ? Nous ne devons pas considérer frileusement, peureusement, les pouvoirs de la science. La seule manière virile de les dominer est d'en mesurer l'étendue et de définir les fondements de notre éthique. Quel type d'homme voulons-nous ? Tous les moyens sont-ils bons pour atteindre nos fins ?

Tout ce qui a trait à l'homme, à sa vie, au meilleur accomplissement de ses potentialités, au pouvoir d'intervention sur son propre protoplasme, va poser des problèmes de choix, d'une exceptionnelle gravité.

L'Institut de la Vie le sait depuis sa fondation. Il se prépare depuis onze ans à ce rendez-vous que l'histoire lui propose. L'Institut de la Vie, intensément, analyse les signes du destin, scrute l'avenir et tente d'engager une réflexion fondamentale.

Cette conférence n'est pas la première. D'autres l'ont précédée, d'autres la suivront. Le mouvement a gagné la terre entière et le mouvement ne s'arrêtera pas car il est porté par l'éternelle aspiration de l'homme à une vie épanouie où serait allégé le poids de la souffrance. Aux prophètes de l'absurde, de la négation et du désespoir, l'événement que nous célébrons apporte un démenti superbe. Vous démontrez que l'avenir de l'homme est ouvert et que le destin se maîtrise à la lumière de l'esprit.

Je voulais vous en rendre témoignage. Merci.

M. Jean CHENEVIER

Je voudrais en quelques mots joindre ma voix à celle du Professeur Marois, pour saluer ici l'impressionnant rassemblement d'hommes de science qui s'est opéré aujourd'hui à l'appel de l'Institut de la Vie, du Professeur Marois, du Docteur McBride. Il est particulièrement encourageant de constater que l'initiative prise par ma Société il y a quelques mois, a donné aussi rapidement naissance grâce au grand désintéressement et à l'enthousiasme du Docteur McBride à cette nouvelle fondation et à l'intérêt quasiment universel suscité par elle auprès de vous Messieurs. Cette initiative ne peut que porter des fruits que notre ami Marois vient de rappeler avec l'enthousiasme et le lyrisme, non déplacés dans de telles circonstances, qui le caractérisent. Je voudrais plus modestement joindre ma voix à la sienne pour saluer au départ, à son départ, la Fondation McBride et lui souhaiter le grand avenir et la grande efficacité que tous nous attendons d'elle.

Monsieur l'Ambassadeur RENOUF

En tant que représentant de l'Australie, je suis très honoré de prendre la parole devant cette assemblée qui couvre un tel nombre de savants célèbres venus de presque tous les continents du monde, réunis pour marquer une de la Vie avec l'objectif de prévenir les handicaps physiques et mentaux. décision prise cette année-ci de décerner le prix de l'Institut de la Vie à mon compatriote, le Docteur McBride. De cette décision tous les Australiens étaient fort fiers. La deuxième initiative est celle du Docteur McBride, lui-même, de consacrer le montant de son prix à l'établissement de cette Fondation 41. De la part du Docteur McBride, c'est une décision généreuse qui révèle d'ailleurs son dévouement à la Science et au bien être de l'humanité, n'importe où elle se trouve dans le monde. Pour sa part, je constate que l'Institut de la Vie n'a pas manqué d'apprécier la générosité du geste du Docteur McBride. Cette appréciation se manifeste en ce moment dans la convocation du Congrès qui se tient actuellement à Paris pour aider le Professeur à réaliser son ambition à l'échelle mondiale en définissant la stratégie à suivre. L'Institut de la Vie a droit à tous remerciements de cette action rapide et efficace. Monsieur le

Président il y avait de très grands hommes de notre temps qui auraient eu un vif intérêt dans le travail qui vise à entreprendre la Fondation 41, le Général de Gaulle et le Président John Kennedy. Malheureusement ils ne sont plus parmi nous. De leur vivant ils ont fait chacun une grande contribution aux problèmes des enfants handicapés.

On peut être certain que chacun d'eux aurait apporté, le plus grand appui à la fondation 41.

Grâce en partie aux efforts de ces deux grands hommes, entre beaucoup d'autres, tel que le Professeur Debré de France, un progrès remarquable a été réalisé depuis la deuxième guerre mondiale dans le domaine des soins et des méthodes d'instruction des enfants handicapés. Il reste beaucoup à faire, mais il reste encore plus à faire dans le domaine qui concerne plus particulièrement le Docteur Mc Bride, l'Institut de la Vie et tous ici présents ce soir, c'est-à-dire, la prévention des conditions de vie avant la naissance de l'enfant; au moins comme vous avez dit Monsieur le Président, 10 % de la jeune humanité s'en trouve menacée. Monsieur le Président, on dit que la prévention vaut mieux que la guérison, mais quand il ne peut être question de guérison la prévention devient impérative. Pour cette raison je félicite l'Institut de la Vie, le Docteur Mc Bride, et vous tous qui avez accepté d'apporter votre soutien à la nouvelle fondation. Je vous souhaite la plus grande réussite dans une œuvre humanitaire si importante. Merci.

Message de Monsieur le Secrétaire Général des Nations Unies

lu par M. BJELIC

Addresse to the participants of the International Conference organized by the Institut de la Vie (Paris, December 1971).

On behalf of the Secretary General of the United Nations, I would like to convey to the distinguished men of science and other eminent personalities gathered here his deep appreciation for your interest in the solution of such important social problems as the role of malnutrition and other factors in inducing birth defects in man.

In seeking to analyze the character of these defects and to pinpoint possibilities for their control, your discussions, organized by the Institut de la Vie, fall within the international concerns of the United Nations which always encourages private effort to deepen understanding of some of the most

complicated problems which challenge contemporary human society. Inherited from the past of emerging as an unwanted result of modern civilization and technical progress, such problems can remain outside of the accepted economic and social priorities for quite a long time — before information media and the public become fully aware of their existence and their real meaning (example : problems of human environment).

Speaking at a recent gathering held in favour of the handicapped by the World Rehabilitation Fund in New York, Secretary General U Thant pointed out that during the first 25 years of the United Nations activities, its social and humanitarian objectives were at times overshadowed by the tremendous political responsibilities which the Organization has had to asssume in trying to keep peace among its member-nations.

However, during this 25-year period various programmes of technical assistance to developing countries, organized by the United Nations and the Specialized Agencies, have contributed considerably towards solving some of the urgent economic and social problems in those countries.

Development of rehabilitation services for the disabled, which started in 1950 is one example of such activities. At that time the United Nations jointly with the Specialized Agencies and in consultation with interested non-governmental organizations, created an international programme for rehabilitation of the disabled. The aim of that programme is to provide advisory services, fellowships, training opportunities, necessary information and other forms of field assistance to developing countries. That programme is still operative and now for the first time there are a number of countries from Africa requesting the kind of technical assistance which has been provided to developing countries in other parts of the world.

We are presently considering possibilities for an international exchange of experience and information regarding the application of modern science and technology in the development of rehabilitation services for the disabled. Some think that modern scientific and technological achievements not only open a new perspective for improving these services but can actually bring about a real revolution in services for the handicapped.

There are also urgent problems concerning prevention of disability to which the United Nations attaches great importance, especially the research necessary to understand and prevent causes of defects. Certain diseases, leprosy for example, can result in chronic disabilities, and we are warned that there might be a link between some eye-diseases and Vitamin A deficiency in the diet of pre-school children in certain developing countries. These factors are additional to those which are the common causes of disability, such as wars, traffic and other accidents.

The United Nations co-operates with a number of non-governmental organizations in attempting to solve these problems and the world organization is open to all constructive initiative towards that end.

The discussion of certain specific aspects of the problem of disability during this conference will, no doubt, help our work on behalf of the handicapped.

ALLOCUTIONS D'OUVERTURE DE LA CONFÉRENCE

Maurice MAROIS

Mesdames, Messieurs, c'est un grand privilège pour moi que de vous souhaiter la bienvenue. Nombre d'entre vous ont accepté de parcourir des milliers de kilomètres. Vous voulez bien consacrer du temps, de la pensée, de l'énergie pour mesurer avec lucidité mais avec espoir les perspectives ouvertes par l'initiative généreuse du Docteur McBride, premier lauréat du Prix de l'Institut de la Vie.

J'exprime à Monsieur Robert Boulin, Ministre de la Santé Publique et de la Sécurité Sociale et à Mademoiselle Marie-Madeleine Dienesch, Secrétaire d'Etat, notre reconnaissance pour avoir accordé leur haut patronage à cette manifestation et pour avoir tenu à se faire représenter pendant la totalité de la durée du Congrès par Madame Farçat et Madame Lefevre-Paul.

J'exprime ma gratitude à M. Denis Forestier et à la Mutuelle Générale de l'Education nationale, nos hôtes, à Jean Chenevier et à la Société Française des Pétroles B.P., au Docteur Charles Mérieux et à l'Institut Mérieux sans qui une telle réunion n'aurait pas été possible.

L'Institut de la Vie est une institution à vocation universelle fondée en 1960 sur deux thèmes d'unité : la science et la vie. Cet Institut n'a aucun caractère politique ni polémique. C'est une œuvre constructive sans autre arrière-plan philosophique que la reconnaissance de la valeur de la vie et de la grandeur de l'homme. Des Comités de l'Institut de la Vie existent dans de nombreux pays. Ils sont constitués par des personnalités du plus haut niveau de conscience et de pensée. Une institution centrale unique pour l'ensemble du monde est actuellement en voie d'établissement.

L'Institut de la Vie organise de grandes conférences internationales sur des thèmes d'intérêt majeur pour l'homme et la société. Le 22 juin dans la Galerie des Glaces du Château de Versailles, le premier Prix de l'Institut de la vie a été solennellement remis à William McBride pour avoir incontestablement le premier dénoncé un cas d'agression flagrante contre la vie : l'action tératogène de la thalidomide sur l'embryon humain. William McBride a décidé de créer un fonds de caractère scientifique pour la prévention des handicaps physiques et mentaux. Il consacre le montant de son prix à ce fonds et il a lancé un appel en Australie, son pays.

L'Institut de la Vie a hautement apprécié la générosité du geste du Docteur McBride et il souhaite l'aider.

Certes, il n'est pas question de nous substituer à tant de laboratoires, d'institutions nationales, internationales, gouvernementales, intergouvernementales et il nous plaît de rendre hommage à leur œuvre scientifique et sociale.

L'Institut de la Vie ne pourra jamais rassembler les moyens d'une recherche à l'échelle planétaire. Son propos est plus limité mais très important. Il pourrait être :

— de définir les grandes voies dans lesquelles la science devra s'engager,

— de soutenir auprès des fondations nationales et des organismes gouvernementaux les équipes de savants qui lui semblent les plus aptes,

— de sensibiliser davantage encore à l'importance de la science appliquée à la prévention, l'opinion publique, les institutions à vocation sociale, les organismes gouvernementaux et intergouvernementaux, etc.,

— de collecter les informations,

— de dépister,

— d'éduquer.

Afin de donner une armature scientifique et une dimension mondiale à l'initiative du docteur McBride, l'Institut de la Vie vous a conviés.

Notre confiance et notre espoir sont très grands.

Les deux premiers jours de notre conférence seront consacrés à la stratégie scientifique. Les six thèmes qui seront traités devant vous et discutés par vous ne sont pas limitatifs. La stratégie institutionnelle fera l'objet de nos débats du troisième jour.

Je déclare ouvert notre Congrès et je prie Monsieur le Professeur Robert Debré de l'Académie des Sciences de nous faire l'honneur de présider cette session.

Robert DEBRÉ

C'est un grand honneur et un grand privilège pour moi que d'être appelé à présider cette première séance de votre réunion. A celle-ci prennent part des médecins et des biologistes de grand renom dont les études sur les grands problèmes dont vous allez vous préoccuper ont une importance majeure. Ce travail concerne un des points capitaux de la santé publique et de la médecine des enfants. C'est le problème des malformations congénitales et des erreurs innées du métabolisme. Maintenant que recule le danger des infections acquises, devient plus important le souci des désordres congénitaux. Grâce à la génétique et aux études faites sur le rôle de l'environnement dans le développement humain, nous pouvons progresser dans ce domaine. On considérait autrefois les malformations congénitales et les erreurs innées du métabolisme comme inévitables et incurables. Une réunion comme la vôtre, l'initiative qui est prise ici signifient que nous ne considérons ces malformations, ces erreurs innées ni comme inévitables ni comme incurables. En effet, on peut aujourd'hui mieux lutter contre ces malformations. La chirurgie des malformations cardiaques, la chirurgie des malformations urinaires, la chirurgie des malformations du tube digestif font d'incessants progrès et nous instituons maintenant des services de cardiologie de l'enfant, par exemple, où il n'y a plus guère de lésions rhumatismales mais où tout l'effort se porte sur le dépistage des malformations congénitales et leur traitement. Les erreurs innées du métabolisme décelées de bonne heure peuvent être l'objet de mesures préventives efficaces; l'arriération phényl pyruvique, les altérations de la galactosémie, l'intolérance au fructose, en sont des exemples.

Des régimes appropriés donnés dès les premiers jours de la vie et suivis attentivement pendant de longs mois ou de longues années permettent d'éviter les désordres secondaires avant tout cérébraux si graves chez le nouveau-né et le nourrisson. Donc la voie est ouverte à la guérison possible de malformations congénitales d'une part, d'erreurs innées du métabolisme d'autre part. Mais cette voie n'est pas la meilleure : la vraie voie est celle de la prévention. Pour cela, il faut essayer de comprendre les causes de ces erreurs innées et de ces malformations congénitales. Or, deux directions s'offrent à nous : d'une part l'étude de l'hérédité qui va permettre de plus en plus de percevoir les risques innés qui dépendent de nos chromosomes et de nos gènes. Ce sont, par conséquent, des études dans ce domaine qui nous permettront d'apercevoir les données et de diriger les consultations d'eugénique en voyant ceux ou celles qui sont porteurs de la tare et qui sont susceptibles d'engendrer des sujets tarés.

Mais ce n'est pas seulement le poids de l'hérédité qui pèse et dont nous aurons à apprécier la lourde servitude, ce sont aussi les conditions mêmes de la reproduction et de la fécondation. En effet, les cellules germinales humaines se modifient : elles vieillissent chez la femme qui a son équipement tout prêt depuis sa naissance. Elles se modifient également chez l'homme et il n'est pas indifférent qu'un spermatozoïde émis depuis quelque temps aille féconder un ovocyte. Il n'est pas indifférent que l'ovocyte soit fécondé chez une femme âgée ou chez une femme jeune. Les conditions mêmes de la reproduction font donc l'objet d'études attentives. Ensuite, à partir de cette cellule et avec une prodigieuse rapidité, se forme un embryon, puis un fœtus. Celui-ci est exposé à tous les risques, à tous les traumatismes, à tous les chocs de l'environnement. Et l'étude de la grossesse avec cette division fondamentale entre les grossesses normales et les grossesses à haut risque doit solliciter au plus haut point l'attention des obstétriciens. Puis vient le drame de la naissance : cette phase difficile où l'enfant abandonne la vie intra-utérine pour « venir au monde », comme on dit.

Pendant cette période, bien des événements peuvent se passer et, vous le savez, l'accident qui trouble l'oxygénation ou l'équilibre du pH atteint les cellules nerveuses d'une façon redoutable au point que le désordre de quelques instants peut peser sur la vie toute entière. Dans les premières heures de la vie, dans les premiers jours de la vie, les conditions dans lesquelles est placé le nouveau-né ont une action décisive.

D'où nos soucis et nos soins pendant cette période de l'existence.

Vous êtes placés, par vos études, à l'origine même du développement de l'homme, mieux encore à l'origine de sa formation, et les travaux qui permettront d'éclairer ce qui se passe dans ces instants solennels, dans ces minutes, dans ces semaines et dans ces mois décisifs, doivent permettre d'éviter une grande partie des malheurs qui tombent sur des familles et qui atteignent un nombre encore trop élevé d'enfants dans nos pays. Je pense donc que vos travaux ont un intérêt considérable et je leur souhaite un très grand succès en félicitant le Professeur Marois, une fois encore, de vous avoir réunis.

Journée du 2 décembre 1971

Première séance

STRATÉGIE SCIENTIFIQUE

PRÉSIDENT ROBERT DEBRÉ

William G. MCBRIDE
The nature and distribution of birth defects in man

Discussions

R. G. WHITEHEAD
The effects of malnutrition on body structure and function

Discussions

M. WINICK
Malnutrition and mental development

Discussions

THE NATURE AND DISTRIBUTION OF BIRTH DEFECTS IN MAN

William MC BRIDE
M. D. F.R.C.O.G.
Sydney Hospital, Harley, Hartsville 2220 (Australie)

Many of you must have wondered why you're here; why did you travel so many thousands of miles at a time of year when you're probably very busy, to come to this meeting? I think you're here in answer to a challenge. I, personally, am surprised that you're here. Exactly one month ago, I was in Paris returning from a World Health sub-committee meeting held at Oxford University and I called to see Professor Marois. At that stage, he hadn't been able to arrange the funds for this meeting. The outlook seemed hopeless. An international monetary crisis was on at the time. Everyone we had approached for funds had very good reasons why they couldn't help us, and I said to Professor Marois : " I think, with four weeks to go, we must call this meeting off. I don't think we can possibly hold it ". He asked me a question. He said : " Are you enthusiastic ? ". I said, " Yes, I am enthusiastic ". He said, " If you are enthusiastic, I am optimistic ". I didn't quite share his optimism. I did, however, approach Quatas, the Australian airline, to see if they could help out in reducing fares. They said they didn't fly to Paris, but they would take it up with some other companies and arrange some international transport. A few days later they told me this could not be done.

A day or two later, I received a cable from Professor Marois saying : " Do not worry. All is arranged. I have arranged the finance ". I thought perhaps there had been a mistake in the translation of this cable, so I put in a telephone call to him and he reassured me that this was the case. I simply do not know how he does it. The amount of energy he has to arrange these meetings, it just bewilders me.

I am amazed that this meeting is actually taking place, and I am delighted to see so many people from so many parts of the world here with us today. I feel that being at this meeting you will not be disappointed that you did make the effort to travel so many thousands of miles in many cases to come to Paris for this meeting.

I come from Australia, which to many of you is on the other side of the world, where people walk on their heads, and is not of any great consequence in international affairs. We have a population of 13 millions, and the state in which I live has a population of 4 millions. I work at the largest Maternity Hospital in the city of Sydney, where 7,000 babies are born every year. It's not the largest in the country : the Royal Womens Hospital in Melbourne is bigger, so we do see a fairly large volume of work.

I might remind you, I hope you won't think I am praising Australia, but it was in 1941 in Australia that Gregg recognised the dangers of rubella, a mild disease in women, but with terrible consequences to the unborn child. In the early 1950's, Dr. Kate Campbell of Melbourne pointed out that she thought that high concentrations of oxygen in premature babies could result in subsequent blindness, and in 1961, it was at our hospital that I discovered the dangers of thalidomide.

Now when each baby is born, I'll just run through this quickly, so I can show you how our statistics are derived : it has to undergo a general inspection, looking out for obvious faults such as cleft lip, mongolism, gross abnormalities such as anencephalus, hydrocephalus, monsterism and cyanosis. We turn them over and inspect the spine, looking for spina bifida cystica. Then they undergo a detailed examination of the face and head, including measurements of the circumference of the head, again looking for cleft lip; looking at the eyes for mongolism, epicanthic folds, protruding tongue, micro-glossia, micrognathia, and again hydrocephalus. We look at the eyes for ocular malformations and for Brushfield's spots. We inspect the mouth for cleft palate. We examine the neck for goitre, branchial cysts, webbing of the neck and sternomastoid tumours. Then we turn to the hands for polydactyly, simian creases, curved fifth fingers, examine the arms for malformations, abnormal shortening and absent or restricted passive movement. We inspect the thorax and detect the site of the cardiac impulse. We auscultate the thorax for cardiac murmurs and bowel sounds, in case of diaphragmatic hernia. We examine the abdomen for epigastric pulsation, umbilical hernias, examine for ectopia vesicae and intra-abdominal tumours, and we count the number of vessels in the umbilical cord. We then inspect the genitalia and hydrocele, imperforate anus and pseudo-hermaphroditism. We examine the anus, for hypospadias, epispadias, undescended testes, inguinal hernia, hips and femoral pulses and look for congenital absence of femoral pulses, coarctation of aorta, exaggeration of femoral pulses (patent ductus arteriosus), limitation of abduction for congenital dislocation of the hip, examination of the feet for talipes and longitudinal plantar crease in Mongolism, and also polydactyly. And we test the baby's Moro reflex. And what does this show us ? Diminution or absence (severe cerebral defects).

We find the incidence of severe congenital malformations in the last two years — in the public Service of the womens Hospital (Sydney) there were 43,000 babies born : severe abnormalities had an incidence of 2 per cent of all births. Talipes requiring splinting, an extra 1 per cent. In addition, there were 4 per cent of babies with mild degrees of talipes which required physio-therapy and perhaps splinting for a few days, but not prolonged splinting as did the 1 per cent of births.

Now the peri-natal mortality of babies with abnormalities, still birth rate was 4 per cent, neo-natal death rate 16 per cent, a total of 20 per cent. So of all the babies born with congenital anomalies, 20 per cent died, either stillborn or in the neo-natal period, so we had a peri-natal death rate of 20 per cent, one in five. We had major physical abnormalities compatible with life of 1.5 per cent of babies, one and a half in every hundred.

Now the table 1 shows the break-down of the twelve most common abnormalities in these 43,000 births. You see, actually there are 13, because we have divided cleft palate, cleft palate where it is associated with cleft

TABLE 1

Incidence of abnormalities 43,012 *babies born women's hospital, Sydney.* 1961-70

	Per 1,000 births
C. Heart disease	3.7
Hypospadias	1.8
Down's syndrome	1,32
C. dislocation hip	0.90
Anencephaly	0.89
Upper limb deformities	0.88
Hydrocephalus	0.70
Meningo-Myelocele	0.65
Multiple abnormalities	0.65
Cleft palate & lip	0.60
Lower limb deformities	0.49
Cleft palate	0.47
Tracheo-Oesphageal fistula	0.23

lip, cleft palate alone. So these are the big twelve in our country. You can see that congenital heart disease heads the list with 3.7 per thousand births : hypospadias is next with 1.8, Down's syndrome 1.32 per thousand : congenital dislocation of the hip 0.90 per thousand : anencephaly 0.89 per thousand : upper limb difformities 0.88 per thousand : this takes in the thalidomide year, we excluded the thalidomide babies, we didn't have many of them, there were only about four in our hospital, but we excluded those to get us a truer representation : hydrocephalus 0.70 per thousand : meningo-myelocele

0.65 per thousand : multiple abnormalities, multiple severe abnormalities, excluding Mongolism (Down's syndrome) with other abnormalities, 0.65 per thousand : cleft palate and lip 0.60 per thousand : lower limb difformities, again excluding thalidomide, 0.49 per thousand : cleft palate, not associated with a cleft lip 0.47 per thousand : tracheo-œsphageal fistula 0.23 per thousand. So these were the big twelve in our hospital.

We did not detect them all, I am sorry to say. We do miss some babies with congenital heart disease. Our examination of the eyes leaves a lot to be desired. We do examine the eyes of babies born prematurely, treated in the premature nurseries, and any other suspected eye abnormalities, but all our babies do not get a thorough routine examination of the eye whilst they are in our hospital for the first week of life.

And then we come to the child at the age of one year. You find that some 5 per cent of children, and this is confirmed by our school medical service which is an extremely good one, 5 per cent of children have got some quite severe abnormalities; — Heart disease, visual defects, blindness, — so that we find that 5 per cent of children at the age of one year or older have got some severe disabilities. These are purely physical disabilities. Then 2 per cent of our children born in the State of New South Wales have to go to special schools because they are suffering from cerebral palsy, 2 per cent.

A recent survey, undertaken by the Spastic Children's Centre in Sydney found that in addition 3 per cent of school children who could go to a normal school, a normal primary school, had mild degrees of cerebral palsy. Children who were unable to write very well, who could not perform fine movements well, were subjected to an E.E.G. examination, and they found abnormal rhythms and asymmetrical E.E.G. in 3 per cent of children attending normal schools. So we get a total of 5 per cent with cerebral palsy — 2 per cent were severe. These children have to be collected in a school bus, a small bus, and taken to the nearest school for spastic children, so that we get an incidence of 2 per cent requiring special therapy for cerebral palsy.

Then, in our New South Wales schools, we find that 5 per cent are unable to undertake a normal school curriculum. When these school children are subjected to intelligence tests at the age of 9, we find that 10 per cent have an I.Q. of less than 80. 5 per cent — half of these — have to have a special syllabus. Schooling in Australia is compulsory and has been for the last eighty years — a child must start school by its seventh birthday, unless it has medical certificates to say that it is unable to attend a school. The majority of children commence school at the age of five years. There are six grades in the primary schooling. They finish primary schooling about the age of 11 or 12, then the higher schooling goes on for another six grades.

They cannot leave school. It is compulsory to attend a school until the age of 15 years. So that 5 per cent have to go to schools for mental retardation. These, in about half the cases, 2 and a half per cent, will, by the time they are 15, will be able to reach approximately a sixth grade standard of education.

That is, they will reach an education standard of a normal 12-year old child. So that we have a total of 5 per cent born with physical abnormalities, 5 per cent with cerebral palsy and 5 per cent with mental retardation, a total of 15 per cent as I'll show later, there will be some overlap in these groups.

Now, major physical defects: even if we put these down at 1.5 per cent, if we exclude such things as cleft lip, cleft palate, which can be treated by plastic surgery, hypospadias, which can be corrected by plastic surgery, we find that of major physical defects we have an incidence of 1.5 per cent.

Severe cerebral palsy: I said a moment ago that 2 per cent had to go to schools for cerebral palsy, severe cerebral palsy, where they will probably not be able to undertake any kind of work except in a sheltered workshop, 1 per cent, and mental retardation where they will never be able to make sure if they have been given the correct change if they buy something in the shops, that 2 per cent with quite severe mental retardation, they can never be thoroughly educated so that 5 per cent of all children born in the state of New South Wales where people are well-fed, well-housed, don't suffer from mal-nutrition, 5 per cent of babies carry a handicap for the rest of their lives.

Just to emphasise that 15 per cent overall. When you take out the overlap, it comes down to about 12 per cent of the children have got either physical abnormality, cerebral palsy or mental retardation, but 5 per cent have a very severe disability. One child in every twenty born.

The table 2 I have just included to show the effects of rubella in women who had suffered from rubella in the first trimester of pregnancy: rubella now should be a preventable disease. The virus has been isolated, a good vaccine is available, but in the days before rubella was preventable in our hospital where pregnancy was not terminated for various reasons, 79 per cent of women had normal children if they suffered from rubella in the first trimester, the incidence was much higher if they suffered from it in the first four weeks of pregnancy than later on. But of the rest of the children, 9 per cent had deafness, 4.5 heart defects, heart and eye defects, 3 per cent, deafness and heart defects 1.5 per cent, deafness and eye defects 1.5 per cent, mental defects and heart and eye defects 1.5 making a total of 21 per cent.

With recent work, we should be able to eliminate rubella as a cause of congential abnormalities.

TABLE 2

Results of maternal rubella (1st trimester)

	%	%
Normal children		79
Affected children -		21
Deafness	9.0	
Heart defects	4.5	
Heart & Eye defects	3.0	
Deafness & Heart defects	1.5	
Deafness & Eye defects	1.5	
Mental defect, Eye & Heart defects	1.5	

Cerebral palsy : Hypoxia in late pregnancy, or even earlier in pregnancy, may account for this high incidence of cerebral palsy. We know this is likely to occur in placenta praevia. If the woman has an accidental haemorrhage or a abruptio placenta as the Americans prefer to call it, late in pregnancy, a prolapsed cord during labour, or are in this group which we call placental insufficiency, and we'll interpret that later in this meeting. So that we know that hypoxia accounts for quite a percentage of children born with cerebral palsy.

Small for dates babies : the baby which goes to term or approximately term, and is very small in size, it has a low birth weight, it, of course, as we know, has a higher incidence of physical anomalies, chromosomal anomalies and mental retardation. So that we know we've got this special group of a baby that we can detect during pregnancy, it's not growing at the rate it should be, it's suffering from malnutrition in utero, or hypoxia in utero, at birth it is small for the duration of pregnancy and this baby is at special risk.

Conclusion

The incidence of children born in this atomic, space-travel era with severe physical or mental handicaps which they will carry, in the majority of cases for the duration of their lives is appalling. Is it not time that some concerted effort was made for the reduction of this human misery ? Although there is excellent work being undertaken in various centres, in teratology, fetal physiology, fetal pharmacology, pre-natal diagnosis, is the amount of work and the amount of research funds devoted to this work sufficient to reduce this disturbingly high incidence of birth defects ? What greater challenge can we be given than to lighten the burden of this human misery by reducing this appalling wastage ?

DISCUSSIONS

R. DEBRÉ : Merci beaucoup, Dr. McBride, de votre exposé qui nous a beaucoup, beaucoup intéressés et qui est la meilleure préparation à la discussion. J'ouvre la discussion. Avez-vous des remarques à faire, des questions à poser au Docteur McBride ?

C. LEVINTHAL :

1. To what extent does rubella — detected or undetected — contribute to your overall figures ?

2. Have you any data on the effects of smoking in producing birth defects ?

W. MCBRIDE : As regards rubella, pregnancy in our country can be terminated if there are medical indications. The majority of women who suffer from rubella in the first trimester we regard as being at risk, since we know that there is about 21 per cent of babies affected, we regard this as reasonable grounds for termination ,and the majority of these pregnancies are terminated. The rubella babies would be included in those figures, those that are not terminated. Unfortunately, in Australia, it is an endemic disease, although at present we have an epidemic. We have an active campaign to immunise all school-girls at thirteen years; any woman who wants to be immunised can be immunised against rubella — I feel that the rubella question is one which we will have eliminated. As regards smoking, if you ask me this in two years' time, I may be able to give you some figures; two years ago, we started to computerise all our obstetric histories; we have a computerised form and on this form, smoking is one of the questions asked of the mother, if she smoked before pregnancy, during pregnancy, how many cigarettes a day... I have read, of course, the work published in England where they feel there is a higher incidence of congenital heart disease amongst the offspring of mothers who smoke during pregnancy; we should be able to produce some quite interesting figures in perhaps a year's time on the subject. We are doing the whole of Australia, in the major teaching hospitals all their obstetric histories are going on to a central computer.

K.N. DEGENHARDT : I am very impressed by the presentation of the data on the incidence of congenital anomalies in the unborn and in the one-year infant.

Australia and West Germany are far distant, but I can say on the background of a large prospective study since 1963 that the incidence of physical anomalies is just the same in our country. In spring this year participating in the first meeting of the European Teratology Society at Cardiff, I got aware that the incidence of anencephaly is ten times higher in Wales than for example in Frankfurt city. We should pay attention to different types of congenital anomalies in different countries. To focuss on etiology of congenital malformation, I may recommend to begin with prospective investigations also in Australia, as it is going on so excellently in the U.S.A. in France (Paris), in Finland, Denmark, Scotland. We have to focuss more attention to the first three months of pregnancy, when the majority of congenital anomalies may be caused.

M. McBRIDE : Thank you, Professor Degenhardt. We have recently undertaken a prospective study. Every pregnant woman that enrolls in our hospital is given a booklet, which is a diary. She is asked to record if she develops any fever, the date she develops it, if she consults a doctor, what the diagnosis is; if she is given any medication at all, she is to record it, if she takes an aspirin for a headache, she is to record it.

D. HSIA : I wish to emphasize the importance of hyperbilirubinemia in newborns. There is considerable evidence of an increased incidence of jaundice in Far Eastern countries not related to G6PD deficiency. It seems that this is seen more frequently in countries with G6PD deficiency though not necessarily seen in association with it.

W. McBRIDE : I should have mentioned, I omitted, we have screened for some five years, every baby born for phenylcetonuria and all jeundiced babies are screened for G6PD deficiency.

T. INGALLS : Is ultrasound, always without risk even if used in very early pregnancy, when the fœtus is very small.

W. McBRIDE : You're probably aware of the recent work that's been done in England, Professor Ingalls, the London group maintain that it is. I myself feel we can't give a conclusive answer at this stage. They published their report, I think, in The Lancet in October, and it seemed convincing, but I agree with you, perhaps it requires further investigation.

P. MARKS : With respect to ascertaining whether there is a relationship between G6PD deficiency and jaundice, screening during jaundiced or early post jaundiced period may not be valid. In presence of hemolysis, enzyme level

will be high owing to young cell population. Probably screening data — to be valid — should be based on studies in infants after 6 months of age.

W. McBRIDE : It's usually three months afterwards, in the majority. They're not screened immediately, but they're followed up of course, particularly the severe ones where they needed exchange transfusions, they are followed up for months, but I think it is at about three months they start to screen them. Do you think that's too early ?

A.A. MOSCONA : Do you have information which would allow you to estimate how much of this high percentage of congenital abnormalities may be due to a genetic background or to environmental factors ?

W. McBRIDE : We don't screen all parents of babies born with congenital anomalies, unfortunately, and I must confess this. Of course, all babies with Down's syndrome, their parents are thoroughly investigated, if there is a persistent family history of anomalies, or recurrent abortions, then they are screened, they do have chromosome counts, but I would think that the majority I use the term " environmental " loosely, are of environmental causes.

C. LEVINTHAL : Does anyone have data on defect frequency in areas where nutrition and medical care is not as excellent as it is in Australia ?

W. McBRIDE : Across the aisle from you, Professor Warkany may be able to enlighten us on this, but I have made several trips to India for United Nations and I feel that in a country like that, where the patients are in hospital for a very short time, Singapore, Thailand, and from what I could see, the babies weren't thoroughly screened and I doubt if I could accept any published figures from those places, but Professor Warkany has made an intensive study of these from various countries, and I am sure he'd be able to help.

J. WARKANY : I'm sorry, I have to disappoint you. The situations are so complex. You are dealing with different ethnic groups, you are dealing with different socio-economic groups, you are dealing with different geographical situations, different medical care, so the figures are not comparable.

R. DEBRÉ : Il faut répéter encore, que les études prospectives sont absolument indispensables et celles qui vont être poursuivies dans les différents pays vont nous renseigner. Nous savons en effet que les malformations congénitales, et l'anencéphalie dont on a parlé tout à l'heure, sont d'une fréquence très variable suivant les groupes ethniques. On ne peut donc pas tirer des

conclusions générales des statistiques capitales qui nous viennent d'Australie aujourd'hui, et les comparaisons avec les différents pays vont être d'une importance extrême pour apprécier des fréquences et, comme on l'a dit, pour chercher l'étiologie. Nous allons donc pour le moment abandonner cette discussion et passer au problème et à l'orateur suivant.

THE EFFECTS OF MALNUTRITION ON BODY STRUCTURE AND FUNCTION

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This conference is centred mainly around the early "formative" period of human life. When I was asked to attend and to give this lecture, I was pleased but a little surprised since my own research work is concerned with the effects of protein-calories malnutrition on body structure and function during the first three years of extra-uterine life. There has, however, been a growing appreciation of the relevance of studies in young children to the nutritional problems of other age groups including those in women during pregnancy and lactation. What I shall do is to discuss briefly my own work in Uganda and highlight the relevance of these investigations to the main subject of the meeting.

In many ways protein-calorie malnutrition is one of the most complex diseases which affects man. It is necessary to have a clear understanding of the pathophysiological mechanisms which are involved in the actiology of the different forms of protein-calorie malnutrition, for this basic knowledge is essential for the development of improved methods of early diagnosis as well as for improvements in other methods for the prevention and treatment of malnutrition.

In both children and in adults there are two main types of protein-caloric malnutrition : Kwashiorkor, which is found in people who eat predominantly high carbohydrate containing staples, and Marasmus, which results when the balance of the different dietary constituents is reasonable but food is available in amounts well below maintenance levels.

The cause and clinical features of nutritional marasmus have been recognised for thousands of years. Throughout evolution, starvation has been a constant problem and the threat of it a decisive factor directing the course of mens' history. Nutritional marasmus can develop in people in any age. The main features are a grotesquely thin person with a wizenned face, protruding ribs and knees and feet which appear disproportionately large on thin legs. There is very little subcutaneous fat and the muscles are very wasted.

In contrast to marasmus the syndrome now known as kwashiorkor has been recognised only relatively recently as a nutritional disease. The name was first used by Cicely Williams [1] in 1935, who stressed primary protein malnutrition as the cause, but not until the 1950's did this concept become universally accepted; many doctors regarded infectious diseases or vitamin deficiency as a more likely actiology.

Much of the earlier literature is concerned with emphasizing the clinical differences between kwashiorkor and marasmus. Growth failure in kwashiorkor is not as marked as it is marasmus and the main abnormality is oedema, resulting in swelling of the feet, lower legs and often the hands, lower back and the face. Gross misery, illustrated by lack of interest and movement together with a feeble whining cry are additional dominant features. Other clinical abnormalities are a fatty liver sometimes accompanied by hepatomegaly, skin lesions and hair changes.

The same syndrome is seen in older children during adolescence and my colleague Dr. J.P. Stanfield has seen pregnant and lactating women exhibiting the same pathological features.

As doctors became more conversant with cases of kwashiorkor and nutritional marasmus, they realised that many mal nourished people did not fit exactly into either category and showed clinical signs of both types of malnutrition. The term protein-calories malnutrition was therefore introduced to describe the complete spectrum of clinical conditions ranging from classical kwashiorkor to classical marasmus. As with so many new concepts, the true purpose of the new terminology was misunderstood and protein-calorie malnutrition began to be used uncritically as a convenience diagnosis, little attempts being made to indicate the specific problems of the individual. It was mainly for this reason that, in 1966, McCance and Widdowson [2] wrote a paper re-emphasizing the fundamental nutritional and metabolic differences between kwashiorkor and marasmus. Their view has, however, not gone unchallenged. Gopalan [3], at a meeting in Cambridge in 1967, categorically stated that in India there was no difference either in the types of food or in the quantities of nutrients eaten by children who developed kwashiorkor or marasmus; there was no evidence that the mothers of kwashiorkor patients had tended to « push » or force-feed starchy foods. Similar sentiments have been echoed recently from increasing numbers of nutritional scientists in other parts of the world.

Thus, it must be admitted that we still do not fully understand why some malnourished people develop along the kwashiorkor pathway and others towards marasmus or along intermediate pathways to marasmic kwashiorkor. However, at the two ends of the protein-calorie malnutrition spectrum, two distinct, clinically-recognisable syndromes do exist. It is essential for us to

decipher the physiological and pathological processes which result in these two syndromes. We must not just talk vaguely about possible effects of malnutrition in general, say on pregnancy and lactation, we must consider the effects of specifically different types of malnutrition.

During the course of development of nutritional marasmus, starvation results in the gradual wasting of muscle and subcutaneous fat and until carried to excessive limits these can be regarded as normal physiological adaptive processes. The body tissues need energy for survival and if sufficient energy is not available from food it must come from the body itself. The wasting of muscle and subcutaneous fat also probably protects other essential body processes as well and the way that this might occur is shown in Fig. 1.

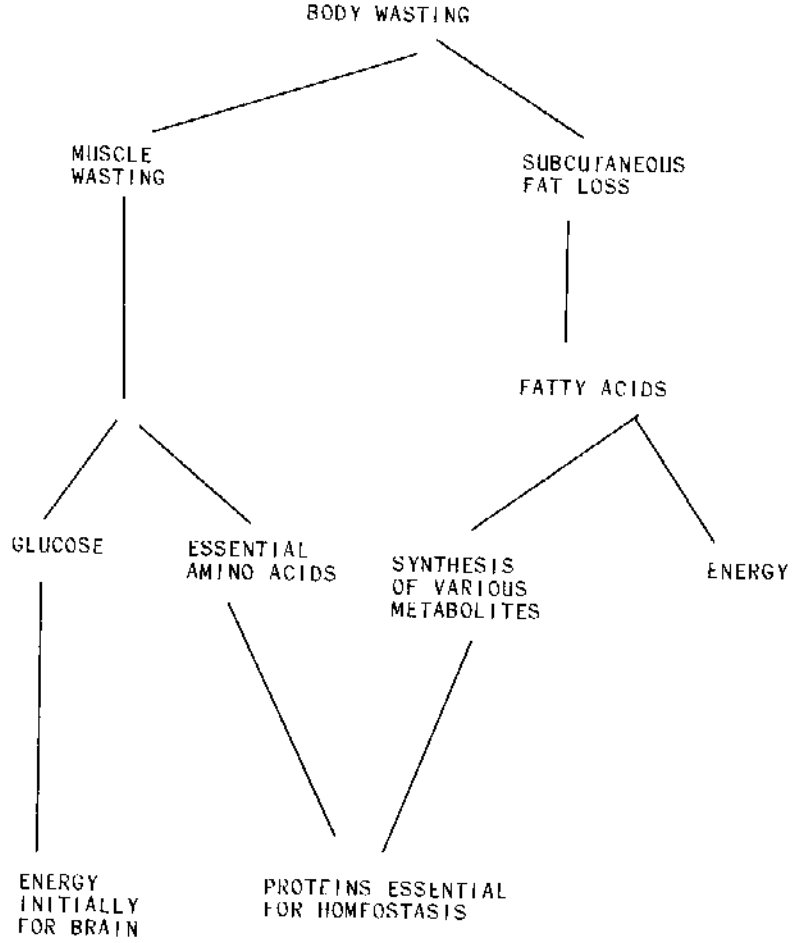

Fig. 1. Basic adaptation to total calorie deficiency. Reproduced from the *British Medical Bulletin* [4]

The breakdown of muscle protein ensures the availability of amino acids for transamination to alanine, the most important of the substrates used for the hepatic synthesis of glucose. Glucose is essential as an energy source for brain metabolism at least during the early stages of total calorie deprivation. Essential amino acids are also made available by muscle wasting and thus, in the marasmic type of malnutrition, serum amino acid patterns and synthetic products ,such as serum albumin and β-lipoprotein can remain in remarkably normal concentration.

In marasmus gradual body wasting is the main feature, metabolic abnormalities and cellular derangement are confined mainly to the terminal stages [4].

During the development of classical kwashiorkor, however, the situation is quite different. Man clearly has metabolic difficulties in adapting to foods with a high carbohydrate content, particularly when the protein content of these foods is low too. This is probably because, on the evolutionary time scale, it is only relatively recently that man has actually cultivated such crops. In young children metabolic derangements can become apparent quickly and lead, in extreme cases, to gross pathological abnormalities such as oedema and a fatty liver. Why a failure to maintain homeostasis should be so prevalent in the kwashiorkor type of malnutrition is still the subject of much research [4].

In people living on the type of high-carbohydrate foods that predispose towards kwashiorkor, there is less need for an accelerated tissue catabolism, since reasonable, although often still limited supplies of energy are available in the diet. Furthermore, the dietary carbohydrate, on digestion, provides the glucose units necessary for brain metabolism and thus muscle breakdown and a subsequent high rate of hepatic gluconeogenensis is not necessary. However, because muscle does not waste so readily and because of the shortage of dietary protein, the free amino acid pools are not nearly so effectively replenished and inadequate amounts of amino acids are available for essential synthetic purposes [4].

The hormonal balance found in such children indicates why these metabolic problems occur.

Fig. 2 shown the fasting serum cortisol levels during the progressive development of kwashiorkor [5]. Except during the terminal stages when appetite is greatly reduced, the cortisol levels are low, sometimes so low that they are barely measurable. This contrasts with the marasmus situation when, as might be expected, the cortisol levels are much higher than normal.

Fig. 3 shows the corresponding fasting insulin levels [6]. For most of the time during the development of kwashiorkor, when the children are eating their high-carbohydrate containing foods, the average fasting insulin levels are higher

than normal. Only towards the end, due to anorexia, do the insulin levels fall in concentration.

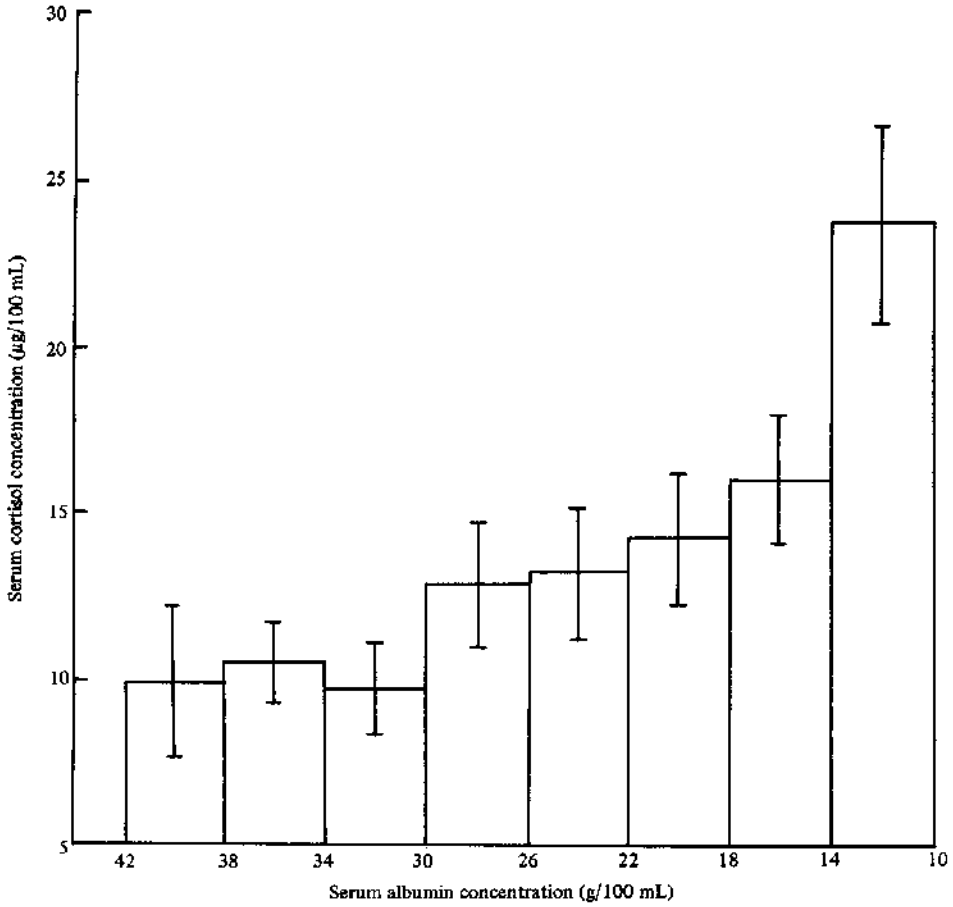

Fig. 2. Changes in serum cortisol levels during progressive hypoalbuminaemia [5].

It might be anticipated that relatively high insulin levels and low cortisol levels would favour the flow of amino-acids out of the serum into muscle cells. In other words, the net movement of amino acids would tend to be in the opposite direction in children developing kwashiorkor as compared with those moving towards marasmus. This is, in fact, what has been observed; during the development of kwashiorkor the serum amino-acid pattern becomes gradually more distorted. Not all the amino acids are affected; the changes are mainly confined to the essential amino acids, concentration of the branched chain amino acids, including valine, being particularly marked.

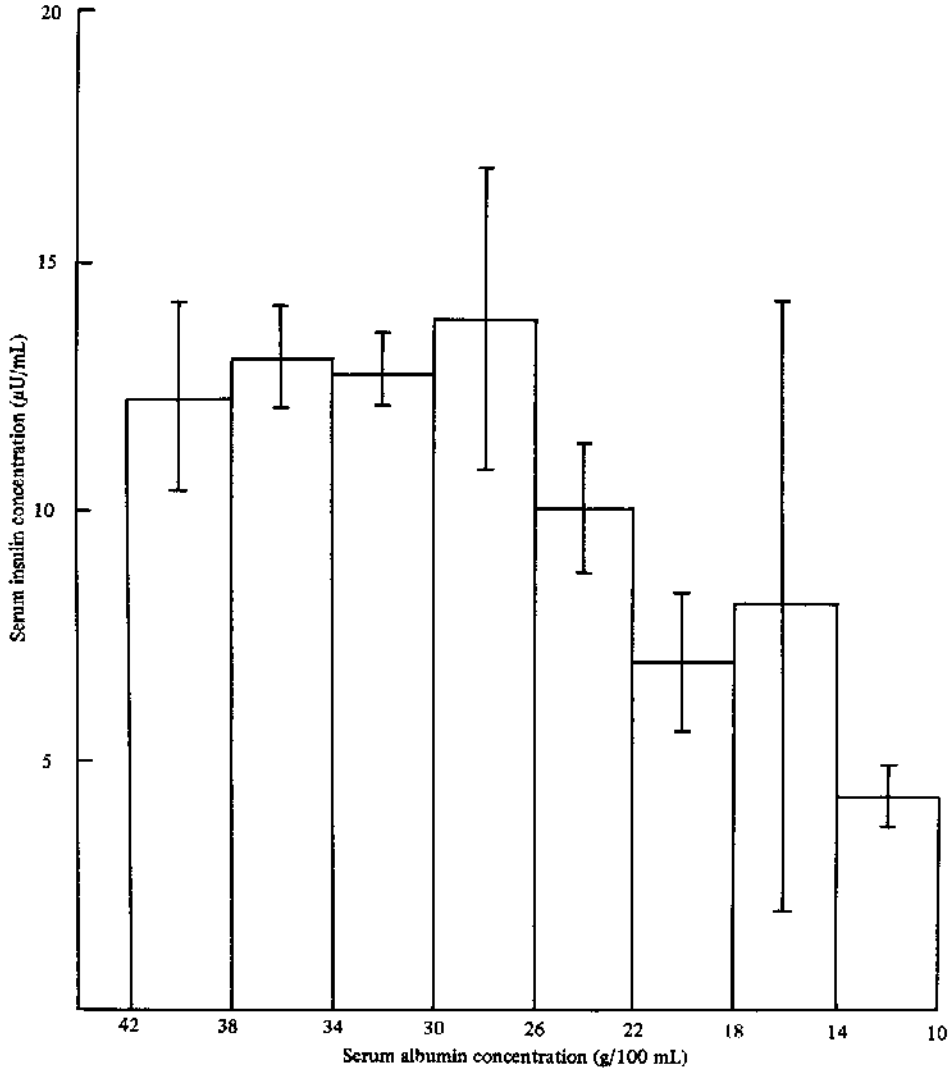

Fig. 3. Changes in serum insulin levels during progressive hypoalbuminaemia [6].

In Fig. 4 the solid line shows the mean valine concentrations in a group of 40 rural Ugandan children studied longitudinally, plotted against age [7]. After 9 months there is a progressive fall in valine levels, the lowest values occurring around 18 months; at this time the children in Uganda are particularly prone to kwashiorkor.

The synthesis of serum albumin is very sensitive to the pattern of amino acids in the serum, and, one might expect a fall in albumin concentration to

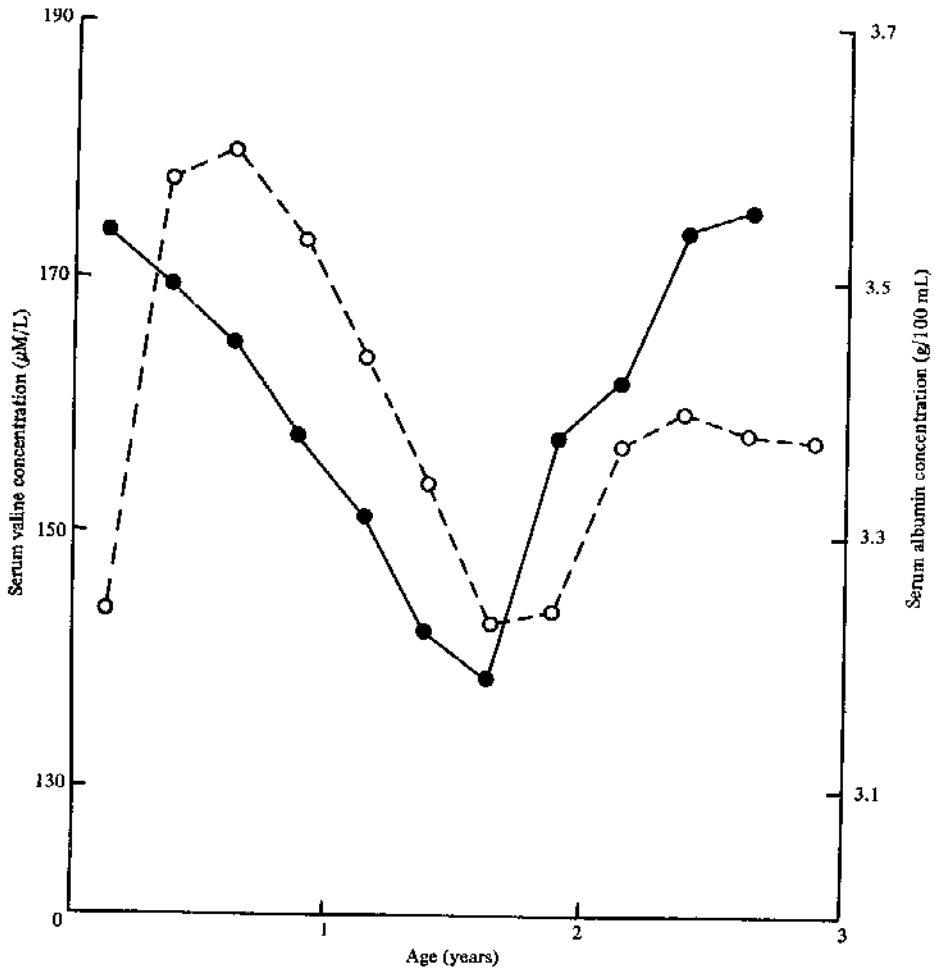

Fig. 4. Changes in serum valine ●——● and albumin concentration o——o at different ages among rural Baganda children [7].

follow closely the diminution in valine levels [7]. The dashed line illustrates the corresponding albumin values. Between 18 months and 2 years the average serum albumin concentration was lower than at any other time. It is also of interest that the rise in average valine concentrations, which occurred after the children had passed the nutritionally « at risk » period, were followed closely by an improvement in albumin concentration.

As the results in Fig. 5 indicate, reduction in albumin concentration are closely followed by falling levels of other circulating proteins such as β-lipoprotein [8]. It is my hypothesis that it is this chronic phase of malnutrition

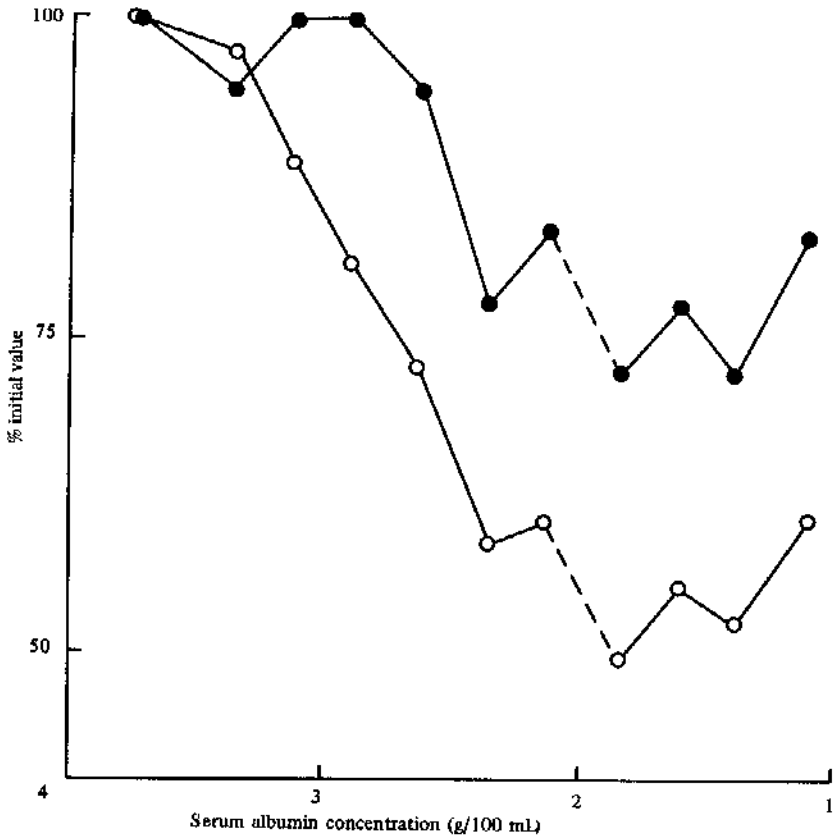

Fig. 5. Changes in serum β-lipoprotein ●——● and cholesterol concentration o——o during progressive hypoalbuminaemia.

when biochemical abnormalities rather than body wasting predominates, which produces the ultimate differences between kwashiorkor and marasmus. I say this because during the acute terminal stages, cessation of growth, further loss of weight and wasting of fat and muscle occur in both types of malnutrition.

In Guatamala it has been shown that the marked faltering of growth in malnourished children at this time is usually associated with infection [9]. In Uganda we have demonstrated that serum albumin levels are also further affected for the same reasons.

Fig. 6 shows dramatically the effects of two series of infections, both on weight loss and on reductions in albumin concentration [10].

In Fig. 7 there are 3 other examples but only showing the albumin concentrations.

Fig. 8 shows the terminal drops in serum β-lipoprotein levels [10].

Symbols for Figs. 6, 7, 8.

Upper-respiratory-tract infections. — Colds, pharyngitis, tonsillitis, non-suppurative otitis media (U), and suppurative otitis media (0).

Lower-respiratory-tract infections. — Bronchitis (B), pneumonia (P).

Gastrointestinal-tract diseases. — Diarrhoea, more than 6 stools per day, possibly plus vomiting and dehydration (D).

Worm infestations. — Ascaris (Wa), hookworm (Wh).

Skin infections. — Mild, such as impetigo and paronychia (I), severe as with abscesses and/or cellulitis (A).

Specific illnesses. — Measles (Ms), whooping-cough (W), herpetic stomatitis (H), malaria diagnosed on the presence of malarial parasites on the blood-film (M), and pyrexia of undiagnosed origin, defined as a temperature of greater than 99.6 °F (37.4 °C) (T).

Nous remercions la rédaction de la revue *The Lancet* qui a bien voulu autoriser la reproduction des figures 6, 7, 8.

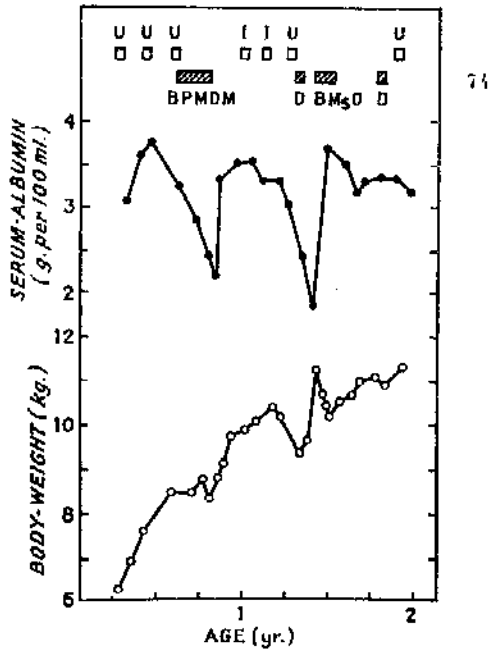

Fig. 6. Relationship between pattern of infection, weight faltering, and episodes of hypoalbuminaemia in a Ugandan child. Reproduced from the *Lancet* [10].

Figs. 6, 7 and 8 all emphasize the point that infection and malnutrition are inevitably interlinked. This is just as true when one is considering a child or other groups at nutritional risk.

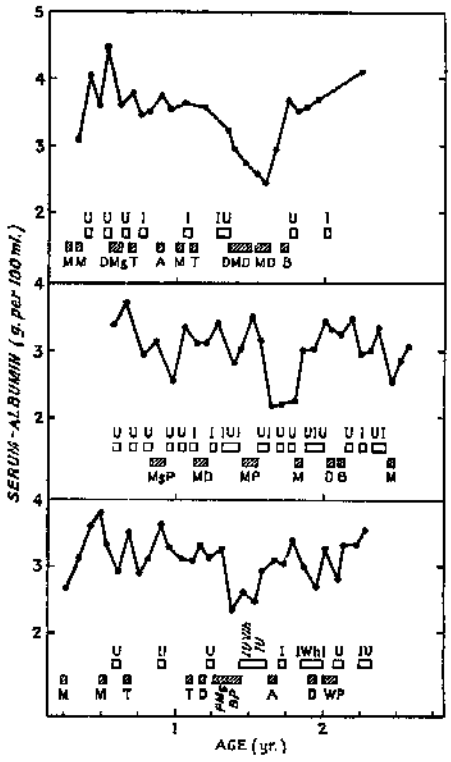

Fig. 7. Relationship between serum-albumin and pattern of infection in three Ugandan children. Reproduced from the *Lancet* [10].

Why do infections aggravate the nutritional status in this way? Many people in the developing countries have diets which, because of their predominantly vegetable nature, are very bulky. In Uganda, a child or adult has to eat about twice the weight of food and about three time the volume compared with a European, to satisfy the internationally recommended calorie allowances. On such a diet even partial anorexia can quickly result in a fall in calorie intake to below the levels required for maintenance. Because of the marginal protein content of the diet, protein intake becomes inadequate too, and, furthermore, not all is available for synthetic purposes; some of the amino acids are degraded and oxidised to fill the energy deficit. Under these circumstances, it is easy to see how infection can aggravate the already existing nutritional problem of the child. This is, of course, of considerable practical importance, any public health programme aimed at eradicating malnutrition should pay equal attention to diet and disease.

The final fall in serum albumin concentration is preceded by a further marked drop in valine concentration as well as in the levels of other non-

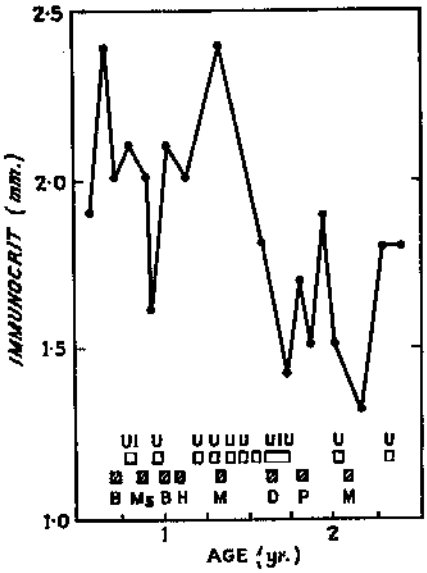

Fig. 8 Relationship between the β-lipoprotein immunocrit and the pattern of infection in a Ugandan child. Reproduced from the *Lancet* [10].

essential amino acids in particular alanine, the latter probably responding to an increased hepatic uptake for gluconeogenesis. It is perhaps surprising that these falls occur especially since cortisol levels have risen at this point and insulin concentration has fallen, but the muscle mobilising effects of these changes on the amino acid pattern is probably negated by a sudden rise in growth hormone levels which would, once more, stimulate the passage of amino acids out of the plasma [11].

The low serum albumin concentrations are associated with an increased susceptibility of the individual to an oedematous episode. The open blocks illustrate the appearance of slight facial swelling, normally called the "moon-face" and the dark blocks, pitting œdema of the legs. Below an albumin of 3.0 g/100 ml, there is clearly a greater risk of œdema (Fig. 9).

The accumulation of œdema is, of course, not just a product of hypo-albuminaemia, factors associated with salt and water retention also play a part.

To summarise : I have traced progressive changes from apparent normality through stages in which the body might be considered at risk to kwashiorkor or marasmus and ultimately to the acute pathological condition. Although these changes might not be the direct concern of this meeting, it was important to discuss them in some detail so that the full range of the effects of different types of protein-calories malnutrition could be characterised for our round table discussion. I will now say a few words about malnutrition and pregnancy.

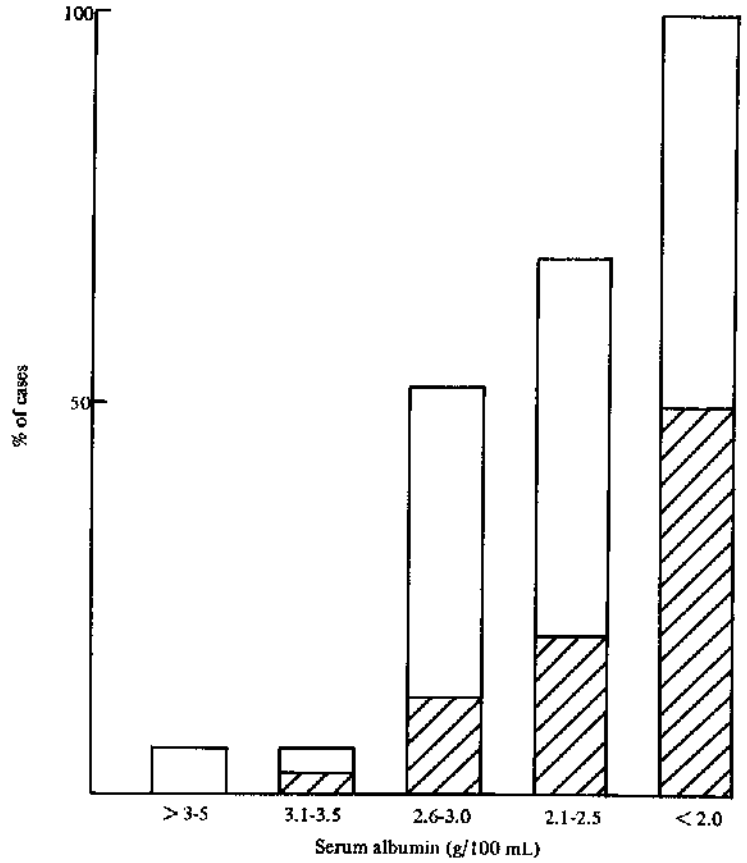

Fig. 9. Incidence of pitting oedema (hatched area) and a moon face (total area) in children with progressive hypoalbuminaemia.

One might expect that severe primary malnutrition in the mother would produce a very serious effect on the development of the fœtus. However, some of the available information indicates that even quite severely malnourished mothers, such as those in concentration camps, can produce offspring who are surprisingly well developed in weight and height at birth. It was birth rate which was affected, not the birth weight. On the other hand, I must mention that Dr. David Morley has convincingly demonstrated that the fourth and subsequent babies in Nigeria are much more prone to subsequent kwashiorkor than the first, second or third offspring. Furthermore, it must be pointed out that the concentration camp mothers were mainly marasmic, they were not suffering from kwashiorkor; there could be a difference between the two types of malnutrition in these effects on fœtal development.

One reason why malnutrition might produce only a small effect during pregnancy is that relatively little extra protein or calories are essential for the formation of the child. For example, the FAO/WHO committee (1965) calculated that the protein content of the products of conception and of the increased weight of the maternal reproductive tissues was about 950 g, which could be met by an increase of only 0.54 g in the daily net utilisation of nitrogen. In terms of the European diet this can be covered by an extra 4-8 g protein/day during the second and third trimester. The corresponding additional energy intake recommended was 200 kcal/day.

Of course many women in the developing countries fail to achieve these extra intakes and the development of the fœtus occurs at the expense of the mother's health. In Guatamala low serum valine levels and an unusually low albumin level was frequently found in pregnant Indian women coming from the lowest socio-economic class. As I have already said, it is not unknown for kwashiorkor to appear at this time.

Even if a baby of only slightly reduced weight is born to a malnourished mother, the subsequent development of the baby will not be normal if her dietary status does not improve dramatically. The average adequate daily milk output required for normal growth of a baby is 850 ml. This has an average protein content of 1.2 g % and thus the total daily loss of nitrogen is 1.6 g per day and this can only be replaced by increasing the dietary intake of protein by some 10-25 g/d, depending on its biological value.

FAO (1957) and the MRC (1968) also recommend an additional allowance of 1 000 kcal/day of energy intake during lactation. It is, of course, known that many women do not increase their energy intake by anything like this 1 000 kcal/day and they can still produce sufficient breast milk, but this is because they are utilising the fat reserves which are built up during pregnancy. The average normal mother has laid down about 4 kg of fat during pregnancy and this can provide about one-half of the calories required for lactation.

Malnourished mothers cannot accumulate these large amounts of subcutaneous fat during pregnancy and often there is insufficient extra food during the lactation period as well. The net result is a diminution in the total volume of milk output.

This has an immediate effect on weight gain. When breast milk output is maintained as 850 ml/day this satisfies all nutritional requirements until a child is 6 kg in weight, but if the volume is reduced only slightly to 600 ml this barely satisfies normal maintenance and growth requirements even in the youngest child. Much lower breast output value are often recorded in the developing countries and when this occurs growth practically ceases unless extra weaning foods can be provided.

Thus, many children in the developing countries are committed to relatively slow rates of growth from an early age and this can be further aggravated by successive episodes of primary and secondary malnutrition as a result of infection. The long term effects of this inhibited growth in early life can be considerable. Follow-up studies in Uganda on children treated for kwashiorkor seen up to 3 years after discharge showed no evidence of an accelerated rate of development, in fact, many of the children appeared even more retarded than they had been during the kwashiorkor episode [13].

A more recent study in Uganda of children 7-16 years after kwashiorkor indicated that they were still small compared both this children of European ancestry and with a control group of Baganda village children with a similar geographical and economic background [14]. Bone age, as assessed on X-ray films of the hands and wrists was 1-2 years behind chronological age.

There is evidence, however, that some catch up does occur following puberty. It is thought possible that growth may continue much later in people malnourished in early life, even until they are 25 years of age. One hundred years ago, Dutch army recruits were still growing between the ages of 18-25 years but today they have achieved their final stature at 18 years.

This brings us to the question of cephalopelvic disproportion, which is relatively common in Uganda and many other developing countries. Until we have more information, discussion of this point must be somewhat conjectural, but many girls probably do have babies before a mature bone structure has been achieved. The magnitude of the problem is illustrated by fact that even among elite Ganda families in Uganda the incidence of obligatory operative delivery for cephalopelvic disproportion is about 12 % . Certainly, Ganda physique predisposes to cephalo-pelvic disproportion but the nutritional origin of this abnormality needs to be proved.

I have elaborated on this particular point because malnutrition may have determined the course of development of a mother's even 20 years before she is pregnant!

I think this point is just as worthwhile considering as the possible effects of malnutrition on fœtus development. It is true that the East African baby, like many other African babies, is smaller at birth than the average European baby; he is lighter in weight, shorter in stature and has a smaller head. But one has to be cautious about using the word malnutrition to condemn the stature of these children. Increased food consumption may produce bigger babies but unless it can be shown that the smaller African baby at birth is at a particular disadvantage, we should not conclude that they are necessarily malnourished. It could be claimed that they have become genetically adapted to their environment, a small head being of advantage when the mother has a small pelvis.

The fœtus can, of course, suffer secondary malnutrition for a variety of reasons and the offspring do tend to be small. For example, the effect of placental malaria on birth weight in Kampala is to cause of 263 g as compared with the weight of babies from non-infected mothers. However, albumin levels in children who have suffered from secondary malnutrition might be normal for that age in spite of the low value found in the mother, a further example of the preferential protection of the fœtus as opposed to maternal maintenance.

Finally, how do we decide when a child or an older person is in fact malnourished. I have already warned against calling someone malnourished merely because diet has made them different. Ideally, only when a change is proved to have significance in terms of whole body or cellular malfunction should the term malnourished be used.

Clearly, in severe cases these stipulations are relatively easy to meet. Until recently, however, there has been little attempt to define internationally accepted standards even for such terms as kwashiorkor and marasmus. The Wellcome Trust working party on Nutrition in Jamaica, 1969, suggested that the crucial diagnostic considerations were the presence or absence of œdema and the degree of body wasting. Using the Harvard weight standards it was recommended that the diagnosis in children with œdema above 60 % expected weight for age should be kwashiorkor but if the œdematous children were less than 60 % weight for age the diagnosis should be changed to marasmic kwashiorkor. Children with weights for age below 60 % but with no œdema were considered to be marasmic.

Defining children who are not obviously ill not so easy. The working party, largely on teleological grounds, suggested that children between 60 and 80 % weight for age should be called under-nourished. This conclusion seemed a reasonable one but there are other considérations, such as susceptibility to œdema. Œdema can occur in children who show very little wasting.

The results I described earlier indicate that serum albumin levels below 3.0 g % in 1-3 year old children should definitely be regarded as abnormal and this would agree with the conclusions of the ICNND committee [9]. Not only does the albumin level indicate susceptibility to œdema, it is also a good marker for such abnormalities as hypo-β-lipoproteinæmia and the risk of a fatty liver and alterations in hormonal balance. However, changes in serum albumin concentration have been criticised as being too insensitive to the earliest effects of manutantion and various workers, including myself, have tried to develop more sensitive biochemical tests. These have included measurements of urea and sulphur excretion on the basis that low nitrogen or sulphur intakes would result in low excretions, hydroxyproline excretion, which is related to growth and to changes in serum aminu patterns. Whilst these measurements are indeed more sensitive than albumin, they can be rather misleading for value

can fluctuate widely in response to recent changes in the diet but they may not always reflect alterations in the nutritional status of the child. In the end we have had to conclude that albumin is the most reliable biochemical marker for the subclinical phases of kwashiorkor [9].

We are at the moment working out the final details of a nutritional health profile which we hope will be a reliable way of defining the nature and severity of different types of sub-clinical protein-calorie malnutrition but so far we have only been concerned with the pre-schood child. Among the different measurements which will be included in the profile are serum albumin, subcutaneous fat loss, degree of musculature change and stunting of growth. The methodology finally adopted for these measurements will probably be a compromise between scientific accuracy and practical feasibility.

The value of our system to other age groups will, of course, need careful, separate evaluation.

Part of this paper is based on an article written for the *British Medical Bulletin* [4] and theauthor would like to acknowledge this fact.

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DISCUSSIONS

R. DEBRÉ : Merci beaucoup pour cet exposé important. Un rapport sur l'Australie, peuple heureux, et nous voilà plongés dans le problème des erreurs innées du métabolisme et les malformations congénitales. Un rapport remarquable sur Kampala, et nous voilà plongés dans la misère de l'Afrique, de l'Asie ,de l'Amérique du Sud, avec la famine et la malnutrition. Ces deux rapports symbolisent les deux situations du monde.

Je voudrais, en votre nom, rendre hommage à Monsieur William McBride pour son étude et son action magnifiques. Je voudrais aussi en votre nom, rendre hommage au travail de Kampala et au Docteur Whitehead. Dans cette université d'Ouganda, une des principales d'Afrique, un effort remarquable est poursuivi depuis bien des années; personnellement nous avons eu, au Centre International de l'Enfance à collaborer avec cette Université : le 10 décembre, dans quelques jours, une grande réunion aura lieu à Kampala pour étudier les vaccinations en Afrique.

Le rapport que vous avez entendu concerne surtout les troubles des enfants une fois nés. Cependant, le problème du retentissement de l'état nutritionnel de la femme enceinte sur l'enfant qu'elle porte a été très bien abordé. Les carences en fer et en vitamines doivent être étudiées à côté des désastres que causent le marasme et le déséquilibre alimentaire. C'est donc avant tout, étant donné le sujet de cette réunion, sur les conséquences pour le développement de l'enfant des troubles nutritionnels que nous voudrions que la discussion s'oriente maintenant.

R. GOOD : Professor Whitehead, You emphasized the intimate association of the influence of infection on malnutrition. Taken in another perspective of theof the influence of infection on malnutrition. Taken in another perspective and viewed not only from East Africa, but from several other areas as well, it has become very clear to us that malnutrition can and does have a profound influence on both specific and non-specific components of adaptive immunity. Galal Aref, a former fellow in my department, has been studying malnourished infants among the Palestinean refugee children in Alexandria in Egypt. These unfortunate children develop at very early age Marasmus, Marasmus-Kwashiorkor. Indeed, they often will have Kwashiorkor as early as 4-6 months of age. You see their mothers are malnourished, breast milk supply is minimal and they are fed rice water or starch water with a little flavouring as their only source of food in early life. Such children, being deprived of protein and

protein-calories early in life, when the immunological system is undergoing its most rapid development turn up with severe antibody formation deficiency and are even agammaglobulaenemic. They have low levels of IgI, IgM not attributable to losses of these proteins, but to deficiency of formation. When such children are in turn fed various diets, they persist even for 6 months — 1 year later to show deficiencies of function of their immunological system. David Jose, a present colleague of mine in Minneapolis, recognised a special relationship of nutritional deprivation and immunological inadequacy in Australia when he was working with pediatric problems among the Australian aboriginal children. Nutritional deficiency in these people is very different from that in East Africa or Egypt and, indeed, takes the form of chronic marasmus. Kwashiorkor is very rare in these children. Infection, however, as well as inadequate school performance are regular concomitants. Jose noted that immunologic functions of these children were distinctly abnormal and responses to certain antigens by antibody production most feeble. By contrast, phytohemaglutinin responses of lymphocytes was found to be quite normal or even increased. Dr. Jose and Dr. W. Cooper and I have been addressing the question of influence of malnutrition on development and maintenance of immunity over the past three years. Our results in rats and mice are consistent and clear-cut. We have found that chronic protein deprivation of moderate severity will inhibit the ability to produce circulating antibodies in mice and rats. With chronic protein deprivation of greater severity, both cellular and humoral immunity are severely depressed. Acute protein or protein-caloric deprivation, even if most severe, by contrast, does not depress immunity but may even increase vigour of humoral immune responses. These profound influences of nutritional deprivation on immunity, I believe, are operating in East Africa and South Africa, because I have looked at the tissues of Kwashiorkor children dying with infections like measles and the overwhelming Hecht's pneumonia and the tissue changes are remindful of the finding in patients with severe dual system immuno-deficiency of genetic nature. I would ask what experiences you have had in relating susceptibility to infection to immunological perturbations in the Uganda children having the different forms of nutritional deprivation ?

R.G. WHITEHEAD : Yes. The point which has been raised is, of course, a very important one, and I am glad that Professor Good has confirmed that different types of pathological responses do occur in different types of protein-calorie malnutrition. He has emphasised what I have been trying to convey which is that we must not just talk loosely about the effects of protein-calorie malnutrition on body function. We must always be specific about the different types of malnutrition.

We have only just started, in my unit, to look at the immune response and so far we have only got some rather elementary results. What we find is that the total levels of gammaglobulin in our malnourished children, are, in fact, considerably above normal. This is probably related to the high rate of infection that they have had.

We are now starting leucocyte transformation studies and here too the preliminary results are not indicating the gross type of abnormal response which we might have expected. But I do not want you to assume from what I have said that the immune response in Kampala in these children is normal; in the rather elementary studies that we have done so far we have failed to find gross abnormality.

In terms of defense against disease, there are other but even simpler things : for example the fact that the skin is much thinner than normal means that it is more prone to change due to trauma, thus the skin is more likely to become infected.

Many of the nutritional problems that our children have are related to the high incidence of infection in the district where they live; because of this, immunisation programmes are just as important as dietary therapy in the prevention of malnutrition. During the course of measles a child loses about a kilogram of weight, and his albumin level may drop by as much as I gram per cent. I have calculated that it costs me, as a nutritionist, more to replace that kilogram and get the albumin levels back to normal than it would have cost for the measles immunisation in the first place. Mr. Chairman, you have mentioned the meeting which is being held in Kampala next week on immunisation. This is one of the points that I shall be making at that meeting.

R. DUBOIS : Nous savons qu'à côté des caractéristiques générales communes à tous les états de malnutrition protido calorique, existent des différences nettes cliniques et biologiques. Pouvez-vous me dire si les modifications des taux de cortisol et d'insuline dont vous venez de parler ont été observées dans d'autres régions ?

Des changements hormonaux semblables ont-ils été observés chez ces femmes, dont vous avez parlé, qui souffrent de malnutrition protido-calorique quand elles sont en âge de procréer ?

Pourriez-vous me dire aussi comment se comportent vis-à-vis des infections, les adolescents et jeunes adultes atteints de malnutrition ?

R.G. WHITEHEAD : Thank you very much. I hope I have understood your questions. Is the hormonal balance different during the development of different types of malnutrition, and the answer is almost certainly yes. This

is why it is so important that we do detailed studies to work out the aetiology of malnutrition in different areas. I have given you two extreme examples, that occurring during the course of the development of kwashiorkor and during the development of marasmus; hormonal balance in these different types of children would appear to be quite different.

You ask about other age groups; whether hormonal measurements have been made among older people as well. In Uganda, the answer is no. We were just starting to make these measurements, but I think your question indicates the interest and significance now being placed on such measurements. In Uganda, studies into *child* malnutrition have tended to cloud all other nutritional considerations. Practically all nutritionists work on malnutrition in the pre-school child; other equally important areas of malnutrition are ignored. The nutritional problems of the pregnant woman, in particular the nutritional problems of the lactating woman, are not being investigated. We are now trying to correct this situation by extending our work into these areas. Many of us who, at the moment concentrate on child malnutrition, in fact now feel that prospective studies into the aetiology of malnutrition should not just start at birth, they should start even before conception. I hope that before not too long we will have more precise information for you.

K.H. DEGENHARDT :

1. What are the experiences in Kampala area concerning brain damage following malnutrition disease in early infancy ?

2. What about the reproductive capacity later on following recovery on malnutrition disease early in life, it means rate of stillbirth, early death rate, congenital malformation rate ?

R.G. WHITEHEAD : I have been asked two most important questions, both at the same time. We have just completed a study on the long effects of an acute episode of kwashiorkor on mental development. These studies have been done on people of about 15 or 16 years, that is, about 14 years after they have had an acute episode of malnutrition. We have used as controls, *people from the same areas,* whom we also studied, about 15 or 16 years ago; these controls had neither kwashiorkor, nor did they show any significant weight faltering during early life.

We have had the same experience which so many other people have had, and that is that when you make a big effort to select adequate controls, you find less difference between the control and the kwashiorkor groups than might have been expected. There are some important differences, but they are not gross. The whole problem needs very careful investigation.

I think there are other factors which are just as crucial as the effects of an *acute* episode of kwashiorkor. Many children around Kampala are living on about 70 per cent of the internationally recommended calorie allowances, yet after 18 months, they are continuing to grow, both in length and in weight, at rates similar to those of British children. I emphasize this in spite of the fact that they are eating less calories than is recommended. Recently we have made a short study to find out how they manage to do this. The answer is that they are apparently compensating for their low-calorie intake by a low energy expenditure. The children spend much less time walking and running than do European children living in the same area. The energy significance of these activity differences is about 25 kilocalories per kilogram. You can calculate that if the children did run around as much as our European children do, then they would not be able to grow and would in fact actually lose weight. Is learning capacity lost by children forced into being less active ? This is an important question which needs to be answered. I think people have tended to be too obsessed by severe kwashiorkor. We must start looking at the overall effects of malnutrition at all degrees of severity.

Now to comment upon your query concerning reproductive capacity. Some of you will know Professor Trussell, who is the Professor of Obstetrics and Gynaecology at Makerere University. This is a topic which he is just about to start investigating. The fact that this has not been investigated before again emphasises what I have already said. In Uganda, kwashiorkor in the 2-year old child has dominated most of our attention. We have been guilty of ignoring other, just a important areas of research. This is one of the reasons why I am so pleased to be here and to listen to your comments and thus get some idea of the priorities which are in your minds.

R. DEBRÉ : Avez-vous quelques notions du rôle sur le développement mental et le développement du cerveau de l'enfant dans le cas de malnutrition de la mère, pendant la grossesse.

R.G. WHITEHEAD : This is again a subject of considerable controversy, and the controversy stems from the rather different results coming from animal experiments as opposed to observations in human beings. The crucial factor seems to be the state of development of the brain at the time that malnutrition occurs. If the brain is in a rapid state of growth, during cellular proliferation, then it is considered that mental development is probably affected. When one carries out experiments on rats soon after birth, in which rapid brain-growth is occurring, then big effects are found; if, for example, malnutrition is introduced during the period when the total DNA content of the brain is increasing.

In terms of the child this would be up to about 9 months. It is also possible that malnutrition can have an effect beyond this time, perhaps in association with the myelinisation of the nerve fibres but it is difficult to be sure. This is a subject in which a great deal of very careful work has to be done.

The effect of malnutrition on mental development is a politically loaded subject. It is very important that scientists working in this area are scrupulous with their scientific design. Many people, for all sorts of dubious reasons, want it to be proved that either malnutrition *does* or *does not* affect mental development. Scientists must be resolute in the maintenance of their scientific integrity.

MALNUTRITION AND MENTAL DEVELOPMENT

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What I would really like to do is carry on from where we left off and concern ourselves with two problems which were brought up during the discussion. The first was the concept of growth, and that is, whether or not in fact, growth is retarded by malnutrition, and whether or not this kind of growth retardation remains. And the second concerns the central nervous system, and that is the growth of the central nervous system. What evidence do we have that malnutrition, at critical periods during development, may or may not affect the growth of the central nervous system ?

Now I think, experimentaly, the data of Kennedy, McCanca and Widderson in the early 50's, suggested quite clearly that if one malnourished an animal from birth until weaning, this animal was smaller at the end of the period of malnutrition and this animal remained smaller throughout the rest of his life, regardless of how the animal was refed. However, previous data had indicated that if one malnourished an animal also during the growing period, but at a later time during development, this animal was also smaller at the end of the period of malnutrition, but that if one refed such an animal, he caught up perfectly well.

So, they introduced, really, the concept of time into this element, the time at which the malnutrition was imposed became important. And what they suggested was that there must be something fundamentally different between early growth and later growth, which allowed the animal malnourished *later* in development to recover in stature, whereas the animal malnourished early in development was unable to recover.

Now, along about the beginning of the 1960's, in Montreal, Canada, Enesco and Leblond introduced another concept. Reasoning that the DNA content of any particular diploid cell of any species was a constant. They used the measure of total organ DNA in any organ which was made up primarily of diploid cells, as a measure of cells number, and then they simply said : " If we know the number of cells, and divide this into the weight, or into the total protein content of the organ, we can get an idea of the

rough size of the individual cell, the weight of the protein content per cell ". And this has been expressed as a protein DNA ratio. Now, using these data, they then examined the growth of the various organs of the rat in terms of the increase in cell number versus the increase of the cell size during growth. And I think the important point to illustrate here is, that if one uses the total DNA content of the various organs as a measure of the rate of cellular growth, the growth of the rat in most roughly stops at around 100 days post-natally, but in every case the DNA content reaches a maximum before growth stops.

Now the time at which this will occur varies from organ to organ. In the brain, in the lungs, it occurs at around 21 days of age; in the heart at around 65 days of age. But in all cases it occurs before growth stops, so that there is a period of growth after which there are no further increases in the number of cells, and in fact, what must be happening is that the size of the individual cell is becoming larger. And if one carefully examines growth, as they did one finds that using these parameters one can define three periods of growth.

During the first period, the total DNA is increasing and the total protein content of the organ is increasing at the same rate, so that the ratio is not changing. We have an increase in cell number or hyperplasia, with no increase in cell size. Then, as a consequence of the slowing down of the rate of DNA synthesis, with net protein synthesis continuing at the same rate, the ratio begins to increase. We have hyperplasia and hypertrophy occurring together. And then, finally, DNA synthesis stops, net protein synthesis still continues, the cells increase in size, and hypertrophy is occurring alone.

They reasoned, therefore, that there would be three phases of growth. Now, given this, one can see what might be expressed as a fundamental difference between early growth which is primarily proliferate cell growth, and later growth, which is primarily hypertrophic cell growth, and one could re-ask the question which Kennedy, McCance and Widderson asked, and that is: " Does this different growth pattern explain the recovery patterns that we described initially " ?

This is an experiment, a very simple experiment, in an attempt to test this hypothesis. One simply takes three groups of rats and malnourishes these animals for the same period of time, 21 days. The only difference is the time at which one imposes the malnutrition. In the first group, the malnutrition is from birth to 21 days, a time when all the organs are in proliferate growth, in the second from 22 to 43 days, a time when all the organs are in proliferate growth except the brain and the lung which are complete at that phase, and in the third group from 65 to 86 days, a time when all the organs are in hypertrophic growth. Now we simply examine the organs at the end of the

period of malnutrition, and after we have totally rehabilitated the animal and he is an adult. In the first group there is a roughly proportional reduction in weight, protein, RNA and DNA content. In other words, the DNA content is reduced, the ratio is relatively unchanged, there are fewer cells of a relatively normal size, and when we re-feed these animals we are left with a reduced number of cells.

Now in the next group again, in all the organs there are a reduced number of cells, except in the brain and the lung. We had the normal content before we started the experiment, there the reduction is in the protein RNA and weight, the ratio is reduced, and if we re-feed these animals we are left with a deficit in the number of cells, and all the organs, except the brain and the lung recover.

And in the next group in every case, the deficit is in the size of the individual cells, the number of cells having been attained before the experiment started, and re-feeding these animals produces a recovery in all the organs. So that for this model, using a rat, one can say that if one interferes with the rate of cell division, then we get a reduced number of cells and that this change appears permanent. Whereas if one interferes with the normal increase in cell size which takes place later, we inhibit this increase, but on re-feeding, the cells simply fill back up and resume their normal size.

But these data suggest something else. They also suggest that the number of cells that any organ is going to have is influenceable by the state of nutrition during the period of time when cells are proliferating and one can produce the reverse experiment which is, again, a repetition of the experiments of McCance and Widderson, that is simply super-feeding animals from birth, for example, until weaning and if one does that, both in the experimental or super-fed animals and after they are adults, both after weaning and after they are totally adults, there is an increase in the number of cells. Within certain limits.

So that, what is being suggested is that within certain limits, one can influence the rate of cell division and the total number of cells in a particular organ, based on the nutrition of the animal during its proliferate growth. Well, this raises a number of questions, especially if one is interested in the central nervous system.

First of all, do the various regions of the central nervous system follow these types of growth pattern? Secondly, is this retardation of cell division common to cells, or peculiar to one type of cell? Now if one studies the rat brain in a regional sequence, and these are very gross regions, the most rapid rate of cell division post-natally is occurring in cerebellum, and it stops at about 17 days post-natally, whereas in cerebrum the increase is slower but lasts

longer and it will level of at about 21 days. In brain stem there is very little increase after 14 days, and in hippocampus there is a discreet increase between 14 and 17 days, and this is put in here to illustrate a point, because this increase is not due to cell division within the hippocampus, it is due to a migration of cells from under the lateral ventricle into the hippocampus which has been shown to occur precisely on the 15th day in the rat.

If we are going to concern ourselves with the effects of malnutrition on the developing brain, we have to concern ourselves not only with cell division, but with cell migration. If one malnourishes an animal from birth, the most profound and the earliest effects are seen with cerebellum at 8 days of age, after 8 days of malnutrition, whereas in cerebrum, the effects occur later, and they are less marked, and the migration of cells into the hippocampus is delayed and perhaps even prevented.

These data and a number of studies which have been done later really tend to illustrate a general principle, and this general principle is that it really doesn't make any difference what region you are studying; what is important is the rate of cell division within that region at the time that the malnutrition is imposed. Cerebellum has the most rapid rate of cell division, and as you can see, is the most profoundly affected.

What about the cell types? Here obviously we can't use total DNA content, but we can do radio-autography and then count the number of labelled cells, and see whether or not there is a difference in the per cent label between malnourished and normal animals. In the rat, — this is post-natal malnutrition from birth — there are no neurones dividing post-natally in the carebrum, and theerfore malnutrition doesn't affect neuronal cell division; the glia are dividing and they are affected.

In the cerebellum, all three cell types are dividing and malnutrition reduces the rate of such a division : under the lateral ventricle the primitive cells are dividing and are inhibited by malnutrition; we think that this explains the reduced number of cells in hippocampus, because these are the cells that will migrate to the hippocampus on the 15th day, and under the third ventricle one sees similar effects.

And again these data, and a number of studies which have come afterwards, would suggest that the cell type also is not of primary importance. What is important is whether or not that cell type is dividing at the time that one imposes the malnutrition.

Now, there are a number of questions obviously raised by this, none of which I can answer completely. One concerns the mechanism by which malnutrition interferes with cell division ,and we have become very interested in this lately and have done a number of studies that have tried to get into

some of these mechanisms. What I would like to do this morning is to show you, not what we are doing in terms of mechanisms because this may be a little too early, but some of the studies which would be relative, perhaps, to our next question and that is, does this in fact occur in the human and what evidence is there for it?

Now, one question which was brought up by Dr. Whitehead is the marker of malnutrition. How do we recognise malnutrition? A number of biochemical markers have been thought about. Now, we have been looking at a certain number of biochemical changes in tissues and wondering whether or not one could mark malnutrition in this way.

Dr. Joan Brazo in our laboratory, for example, has been looking at the enzyme DNA polymerase, in an attempt to know whether or not that enzyme varies with the rate of cell division, so that with one single enzyme analysis in a tissue, one could get an idea of the rate of cell division in a whole brain, if one plots the rate of cell division, in green, and one plots the activity of DNA polymerase in red, they superimpose.

If one breaks this down to the fore brain, which has a different rate curve, one gets a different rate curve of activity for DNA polymerase which also superimpose.

If one does this for carebellum, where there are two peaks in DNA synthesis post-natally, — one occurs early, and one occurs later — one gets two peaks in enzyme activity, again correlating with the rate of cell division. So this and further studies that Dr. Joan Brazo has done in other tissues would suggest that the activity of this enzyme in fact does correlate quite well with the rate of cell division. Now, if one malnourishes an animal and looks at the activity of this enzyme, in malnourished versus normal animal, and if one expresses it per cell per milligram of DNA, or per milligram of protein of specific activity, one finds that the activity activity of this enzyme is reduced. Therefore it may provide then a marker for studying nutritional effects on the tissues, in terms of these effects on cell division.

We have also, we and many others, been interested in RNA metabolism in malnutrition, because RNA metabolism has been shown to be markedly altered. Another enzyme that Dr. Pedro Rosso in our laboratory has been looking at, is the enzyme RNAse. Whether or not this enzyme has any physiologic significance we can bring up later, but the point is that if one studies this enzyme, one finds some interesting things about enzymes in general. If one expresses the activity of this enzyme per milligram of DNA, per cell, the activity of this enzyme increases through development. If one expresses the same enzyme per milligram of protein of specific activity, it falls during development. And if one expresses the activity of this enzyme per milligram of RNA, per milligram of its substrate, it never changes during development.

These data are indicating to us that the activity of this enzyme is increasing per cell, it is increasing less rapidly than other proteins are increasing during this period, but at the same rate that its substrate is increasing. And these data, then, have sort of made us lean to the view that this enzyme might, in fact, have something to do with RNA metabolism.

But if one then malnourishes an animal, and this is the point that I want to make, and then studies the activity of the enzyme, using any of the three measures of activity there is an increase in the activity of this enzyme in brain. So that here is an enzyme, then, which during malnutrition does the reverse of DNA polymerase, it increases in activity. And this enzyme is, in a sense, important to us as a marker, because this enzyme is present in serum, it is excreted in urine and can be found in amniotic fluid in the highest amount that so far we have been able to demonstrate this enzyme in any body tissue.

Considering these data, what kinds of analogies can we draw to the human situation ? First of all, we can ask the question : does the human brain grow quantitatively in the same way, or qualitatively, I should say, as the rat brain ? The protein content of brains of children in New York City who died of accidents, poisonings, crib deaths or some catastrophic event but who were normal in every other way (this is a catastrophic death) and of brains from prenatal life (these were therapeutic abortions) up through the first year of life shows a linear increase.

In contrast, if one studies the total DNA content of such brains, there is a linear increase up to about birth, then it begins to level off and by about a year of age — and now with more data it may look as late as fourteen months of age — we have got all the cells we are going to have. We have reached our plateau of DNA. So that qualitatively the human brain is growing in the same way as the rat brain, that is, that all growth after this is due to an increase in cell size, rather than in cell number.

If one looks at the various regions of the human brain — in fact, the qualitative differences are different in the rat thank goodness — here the most rapid rate of cell division is in cerebrum and that all three regions stop at about the same time, at about one year of age. These data suggest that when we're interested in the effects of malnutrition on cellular growth of the brain, one should study the effect of malnutrition during the first year of life, and perhaps before.

This is a baby similar to the baby Dr. Whitehead showed you, a typically marasmic baby. Now these data which I am going to describe, were collected in Santiago, Chile, in a combined study. And this baby, when compared with a normal one a,s was pointed out, is smaller, emanciated-looking, has a reduced

head circumference and shows all the stigmata of marasmus. New when such a baby comes in hospital, one of two things can happen : either the baby can succumb to the disease, in which case one can study the parameters that we talked about, or the baby can recover, in which case one can rehabilitate this baby making maximal nutritional input, send the child home under very careful nutritional conditions, send a nutritionist to the family, and then study the development of such a baby.

All the children who died with marasmus had a reduced weight of the brain, which is the same kind of data which had been found at other places. Not only was the weight reduced but the total protein content of these brains was reduced, so that this was a dehydrated brain. The total number of cells, the DNA content, in such brains was also reduced. So that studying whole brain, in severe marasmus occurring in the first year of life, shows that there are a reduced number of cells in the brains of children who died of this.

In a subsequent study, in which the regions were looked at, there are a reduced number of cells in cerebrum. The same is true in cerebellum, and it occurs quite early in cerebellum in contrast to cerebrum; in fact, even in brain stem in the human there are a reduced number of cells.

So that I think we can say that in this form of severe malnutrition in the first year of life, one does get an inhibition in the rate of cell division and one gets fewer cells.

Now what can we use as a measure of these changes ? Head circumference has been used for a long time as a measure of nutritional status and of brain growth. And it was possible for us to correlate these kinds of changes with changes in head circumference.

Before I do that, let me just take up one other point and that is that it has been shown in rats that myelination is also affected by severe malnutrition. We were able to look at some of the brains of some of the children who died of malnutrition during the first year of life in terms of myelination, measuring for example total brain cholesterol which in the human also is reduced, as in the rat. The total phospho-lipid content is also reduced. A point which again has been shown in animals, but is just as true in the human, is if one expresses a lipid DNA ratio, in other words, the amount of lipid or myelin per cell, this remains relatively constant if the malnutrition occurs during the first year of life. In other words, the rate of cell division and the rate of lipid deposition are being inhibited at about the same rate. But then, cell division stops, myelination continues beyond this, and the lipid content per cell begins to drop.

Let me go back to the question of the measurement of these parameters using head circumference. In our children, just as in children throughout the

world, the head circumference is reduced. This reduction is very severe but, after all, these are children with the most severe types of marasmus, these are children who died of their marasmus, and if one correlates the weight of the brain with the head circumference, this follows the normal curve. In other words, this head circumference is adequately reflecting the weight of the brain in this marasmic infant. The same is quite true about the protein content of the brain, and the same is true about the DNA content, except for three cases in which the DNA content is actually more reduced than the head circumference, and we'll go into those three cases in just a minute.

So then, these data would indicate that for this kind of severe malnutrition, severe enough to cause death, I emphasise this, the head circumference is a relatively good measure of these kinds of changes that are occurring in the brain. Now, what happens if these children survive? The data are not different from the data of Birch and Cravioto and of many others in children coming from poverty afflicted populations who have survived a bout of marked malnutrition during the first year of life. Belonging to the same kinds of children whose brain we were able to study, most of these children fall into an educable group, but that a high percentage of these children are simply trainable and that some small percentage have to be institutionalised. So that there is retarded development using the Birch and Cravioto criteria in this particular group as in their studies.

If one summarises these things, and if the maramus is severe enough to cause death, cellular changes will be found in the brain; these cellular changes will correlate best the reduced head circumference, they will still correlate but less well with the reduction in body length, and they will not correlate at all with the reduction in weight. And then, if the child survives, there's likelihood of functional changes, and these functional changes will correlate in severity with the reduced head circumference, less well with the body weight, but they will still correlate, and not at all again, with the ultimate weight.

Those cellular changes which have been investigated in the human brain secondary to severe postnatal malnutrition, are the same as those which have been found in the rat brain. But these data open up another question which was alluded to earlier and which I would like to conclude with. And this is, what about the fœtus, whose brain is in the proliferate phase? Does malnutrition, or can malnutrition affect proliferation within the fœtal brain? Or is the so-called « small for dates » or fœtally malnourished baby a baby whose brain has been affected? Well, the first point I would like to make is that I feel very strongly that the fœtally malnourished baby, is not a single entity. This is a very, very mixed entity with many, many different components. Let me illustrate this in animals. For example, if one studies the

placenta, which is an organ which grows very much in the same way as other organs, cellularly, and if one ligatures the uterine artery in a rat at the 14th day of gestation, and then looks at the changes that are occurring in placenta, one produces very predictable changes. This occurs during cellular proliferation. The weight, the protein, the RNA and the DNA content are reduced, the number of cells are reduced, the size of the cell is relatively unchanged. What we would have expected. If we study the fœtus after such a clamping, we find that we product growth failure of 67 per cent of the weight, but that this is a growth failure which is asymmetrical as far as the organs are concerned. In fact, the brain is almost not affected at all, the DNA content of the brain being perfectly normal, whereas the liver has a 45 per cent reduction in DNA content, as does the lung. So here we have produced growth failure *in utero* in a very asymmetrical way and we have spared the brain, using these parameters of measurement.

If we use tissue RNA as a measure, within 24 hours after the ligation one can show an increase in the activity of this enzyme in the placenta, which continues up through 72 hours and if one looks at the fœtal brain and liver, after 48 hours there is an increase in RNAse activity in the brain; it is the only change we have been able to demonstrate in the brain with the procedure. In liver it occurs more markedly and earlier. The point that I am making is that the brain is relatively spared, at least using the gross techniques that we have, except for this one technique which may bring a subtle change and may have meaning or no meaning.

In contrast to this, if one produces fœtal growth retardation by protein restricting the mother, from the 5th day of gestation until birth, one produces relatively predictable changes in the placenta, that is, a reduced number of cells, with a relatively normal cell size, and a paradoxical in the RNA-DNA ratio, which we have seen in a number of cases and which I won't go into right now. But in any event, the number of cells are reduced, the size of the cell in not affected. Here, however, if we look at the embryo, at 16 days of gestation, we find that in every area we look at there is a reduced cell number in the brain, both in grey matter and in white matter, and at birth we find that again we've got a smaller animal but there is a symmetrical reduction in all the organs and the brain is affected with a 15 per cent deficit, just about the same as all the other organs. But perhaps even more significant is that here again we have this prenatally induced 15 per cent reduction at birth, and you may remember that if one malnourishes an animal from birth to weaning, one gets about a 15 per cent reduction, but if one malnourishes an animal from conception until weaning, during the total period of time when cells are dividing in the brain, we get a 60 per cent reduction in cell number. So we have an increase which is greater than the

sum here, the duration of the malnutrition during this proliferate phase becomes very, very important under these circumstances.

The measurement of free RNAse in plasma of children who are severely marasmic, shows an increase of activity which falls off relatively rapidly with therapy. Now we need a lot more data, before we can know what this means, or if, in fact, it is going to be valid in a lot of cases, but the point that I want to make here is that RNAse may then be a useful tool for assessing nutritional status and the rate of recovery. And the reason I bring this up is because, as you saw, RNAse increases in the rat placenta when one does this kind of a clamping, and in human placenta, if one uses the normal kinds of measurements of cells that I talked about originally, one does find that severe malnutrition does affect cellular growth of the placenta. For example : in children with fœtal growth failure, it has been shown that the placentas have a reduced number of cells. Simply looking at the indigent population of Santiago, Chile, a population that would be prone to malnutrition, one found that half of the women's placentas had a reduced number of cells. And in a study carrier out more carefully by Dayton and Filer in Guatemala, in which malnourished women's placentas were looked at, all of the placentas, 20 out of 20 women, had a reduced number of cells. And here is a single case of anorexia nervosa in which a woman became pregnant, went from 80 to 84 lbs during her pregnancy, gave birth to an infant under 2,000 grams at term : her placenta contained 50 per cent of the number of cells that one would expect. So, severe prenatal malnutrition in the human apparently will affect the rate of cell division in placenta; in a group of women that we studied in Quito, Ecuador, the RNAse activity of such placentas in malnourished women is again, quite markedly increased, when compared to normal. So I think the data are beginning to accrue now, that severe malnutrition during pregnancy will affect certain cellular growth parameters in placenta.

What about human brain ? We don't have any direct evidence in the human brain at the present time, but I think there are some indirect evidences which at least we ought to be aware of. If one takes all the children whose brains have been studied in these terms and who died of malnutrition, and if one puts them together, one gets three groups : in the group of children with kwashiorkor, and these are children who died generally in the second year, children we studied in the second year of life, their malnutrition not occurring till after the first year of life, the DNA contents of the brain has been normal, the ratios have been reduced. In those children who died during the first year of life, they all have a reduced DNA content, but one group has a reduction of about 15 per cent, and a second group has a reduction of about 60 per cent and the difference between these two groups it that in the first group they were all of normal birth weight, over 2 500 grams, whereas in the other group they were all

below 2 000 grams at birth, suggesting one of two things. Either these were prematures, and the premature infant is more susceptible to the effects of postnatal malnutrition, or in fact this is the clinical counterpart of the double deprived rat that I showed you, and this child had already suffered inter-uterine malnutrition and then had super-imposed on it the later effects of postnatal malnutrition.

To summarize : from a cellular point of view, one can say that severe malnutrition postnatally will affect cellular growth of the rat brain and the human brain, that prenatally certain types of malnutrition — protein-restriction of the mother — will affect cellular growth of the rat brain, and this type of malnutrition will affect cellular growth of the human placenta, and that there are some data which, at least at the present time, make it incumbent on us to look more closely at the effects of this kind of malnutrition on the human brain. Now, what we are talking about, in conclusion, are cellular changes, changes in cell number, cell size and in some enzymes. And I don't at all want to leave you with the impression that we feel that these changes have anything to do with the kinds of functional changes which have or have not been described in the malnourished human. There are no data to indicate that any of the functional changes, if in fact they do occur, are caused by any of these cellular changes or any of the other changes, chemical changes which have been described in the brains of malnourished infants or animals.

DISCUSSIONS

R. DEBRÉ : Je voudrais dire, seulement avant d'ouvrir la discussion, que j'ai été interrogé récemment par une fondation américaine qui demandait des conseils pour l'attribution de ses fonds à des recherches pédiatriques; et j'ai répondu, que l'un des problèmes les plus intéressants est l'étude du cerveau en liaison avec la nutrition de la mère et du petit enfant. Il faut poursuivre les travaux dont nous venons d'entendre un exposé très important.

V. INGRAM : Could the observed increase in ribonuclease be due to release from dying cells and could it therefore be taken as evidence for cell death during malnutrition ?

M. WINICK : Well, I would not go so far as to interpret it as evidence for cell death, but we have certainly considered it, and feel that this is quite a possibility. And I have suggested to one of our post-doctorate Fellows that he might want to look into something like coronary artery, disease in serum, so I think it is quite possible that what you are saying is true. We do feel in fact that the changes in ribonuclease are quite non-specific. We don't think that they are specific to malnutrition at all, but we think that in a malnourished infant whom we have to follow in terms of what therapy is doing, it might be a useful index. That's really as far as I'd go.

C. LEVINTHAL : Could you explain your sceptical remark concerning functional changes associated with malnutrition ?

M. WINICK : There are certain things about which I am not at all sceptical, and there are other things about which I am : I think it is fair to say that malnutrition in association with the whole complex of the poverty situation these children come from, causes retarded development. I don't think anybody would argue that point. The question which is may be simply academic, is whether or not malnutrition itself, if it were operating in a vacuum, could cause these changes. Now, none of the studies which have been done are able to isolate malnutrition into such a vacuum. But attempting to deal with all the other variables in a number of retrospective and prospective studies that is how which have been done, at least I interpret these data, it would look as though malnutrition alone is a significant cause of this retarded development. And moreover, the time at which the malnutrition occurs seems to be extremely

important. Cravioto and Birch's data would indicate that the earlier the malnutrition occurs postnatally, the more likely the changes induced functionally are to be permanent. Now, recently a sort of fly has been introduced in the ointment, and that is in terms of the recovery : whether or not malnutrition is acting directly, or whether or not it is acting in another way, and that is, could it be that the malnourished child is unable to receive from his environment the normal kinds of other stimuli which are provided during development, and could it be a general lack of stimulation then which is causing these changes, and is it possible then that if one enriched such a child's environment after he was malnourished, we could reverse some of the functional changes which have been induced by malnutrition. In animals, there are data which would indicate that these kinds of behavioural changes can be reversed by certain kinds of enriching procedures. In humans, at the present time, I know of only two studies in which this has being tried, one is in Cali, Colombia, and one is in Santiago, Chile, and I don't think there are any data at the present time which indicate whether one will be able to reverse this. From my standpoint I think this is a fair interpretation of the data. Then if we go any further in attempting to interpret the data, if we attempt to say these kinds of cellular changes are the cause of the types of functional changes which are being described, then I certainly think we are way out on a limb and we really don't have any right to say those things.

A.A. MOSCONA : Is it known if there are significant changes in the development of the vascular system in various regions of the brain as a result of malnutrition during prenatal and early postnatal developments ?

M. WINICK : I don't know of any such studies. Maybe Dr. Whitehead does. Some relatively careful histological studies have been done, but I don't know whether even the studies of Platt have reported specifically on the vascular system, and I am certain that no other kinds of studies have been done. So I don't really think it has been adequately studied. But you've got a reason for asking that question, and I'd like to know what that reason is.

A.A. MOSCONA : The reason is in terms of the measurements of DNA, which would obviously be affected by the amount of vascular supply.

M. WINICK : Well, I agree. I think that as to the reduced DNA, — this is not saying that there is no reduction but in cells in the vascular system, but if there were a selective reduction in the vascular system, to the extent that was the only thing that was being affected, — then I think one would see this in the kinds of histological studies that we have done. Moreover, when one does

a differential counting of glia and of neurones, one is not counting vascular cells, and one gets roughly the same result, so I think that that's a reasonable point, but I don't think that the vascular system is being selectively affected. There is no evidence that this is so.

A. MONROY : Dr. Winick, you seem to equate incorporation of thymidine in the nuclei of nerve cells and cell division. Is there any evidence that in the nerve cells, e.g. in the Purkinje cells, there is the same DNA content from the neuroblast to the fully differentiated nerve cell ?

M. WINICK : That's not a very naive question; first of all there are data, in the central nervous system, both in cerebellum and in some areas of cerebrum, indicating that Purkinje cells in fact are tetraploid, not diploid. So, that's the first thing. The time when they become tetraploid during development, I don't think has been worked out. Now, the question that you ask is a very important one, and that is : can we really use DNA as a measure of cell number if there is a significant amount of ploidy occurring within the particular tissue ? First of all, from the standpoint of the brain, I think that the number of tetraploid cells are so small in comparison to the diploid cells, — if, in fact, this were true that it would induce a very small error. But the other point that you raised : what about an organ which, for example, has more tetraploid cells, say, like the liver ? The important thing in terms of using this concept, and this was pointed out by Enesco and Kahn, is not really whether or not the cell is tetraploid, but whether or not, first of all, malnutrition or whatever you are studying changes the ploidy and secondly whether or not the ploidy is in a state of changing at the time that you are making your study. So that one can use the total DNA content of the liver, if one studies it at one point in time when ploidy is not changing. In the brain we do not know when, if ever, the ploidy of these Purkinje cells becomes tetraploid. But, as I said, I don't think we are dealing in the brain with an order of magnitude which is large enough to introduce too great an error here.
K. HIRSCHORN : In rats with permanent reduction of brain cell number, is there an effect on such noncultural intelligence tests as maze-learning ?

M. WINICK : I can answer your question, and then I am going to push it a step further. The reason for it is that I don't want us to draw the wrong conclusions. If one does this, and it has been done, a number of psychological tests in rats have been done, and if they do show behavioural deficits, for example, they would show a decrease in exploratory behaviour during the time that the malnutrition has been imposed, which will persist for the rest of their lives, they will show abnormalities, not in maze-learning, but they will show abnormalities in extinguishing certain conditioned responses, and this is

true not only in rats, but it is true in pigs, and it has also been described recently in monkeys, then I think there is no question that behavioural changes can be induced by malnutrition and can be induced permanently. But I don't want to leave you with the impression that these changes have anything to do with the cellular changes. Because, for example, if you super-feed rats from birth until weaning, Francova and others in Czechoslovakia, have shown that these animals also show behavioural deficits and we know these animals have an increased number of cells, and in fact, the behavioural deficits are very similar to the kinds of behavioural deficits that are shown by the under-fed rats. So that this is a very complex area we're in.

D. HSIA : Dr. Paul Wong has found that after the injection of single amino acids to fetal or newborn animals, there is disaggregation of brain polyribosomes to monosomes and reduction of protein synthesis as measured by C14 amino acid incorporation. This does not occur in older animals. This occurs with certain amino acids like phenylalanine and branchained amino acids, but not with glycine. This may be related to excessive amino acids in certain inborn errors of metabolism and their mental development. Imbalance of amino acids may also be important for brain development in general.

M. WINICK : Yes. I am aware of the work that you are referring to, and I think we ought to take it in context with the work of Monro and of others in terms of the effects of a reduced protein or amino acid mixture, and the effects produced on polysome aggregation which are precisely the same as the effects which Dr. Wong has reported as increasing. And so I think the question does come up whether an imbalance is important, or whether or not what we are looking at is, in fact, a very non specific reaction. Because if one increases phenyl alanine content in the blood of animals, one produces cellularly, in terms of the rate of cell inhibition, exactly the same effects as one gets with the occurrence of malnutrition I talked about, and the same experiment can be done with galactose. And the thesis I would like to bring forth, really, is that we are not looking at a terribly specific reaction here. What we are looking at, is a reaction or rather a non-specific reaction which is very specific in terms of its time-dependency, and secondary to this kind of an insult during proliferate cell growth, one gets interference with the rate of cell division. One can do this : for example, — and somebody brought up a question about smoking before — there are data, Howorth data from Canada, which would indicate that if one smokes rats — and there are ways of doing this — when they are pregnant, one can show that there is a reduced rate of cell division in the placentas of such rats, and in the brains of the fœtuses. So again, in using this kind of a measure, one is getting a response based on the time when the stimulus is imposed. Dr. Wong's study would indicate certainly that one can

do this by increasing the contents of certain kinds of amino acids, which I find really fascinating.

O. HECHTER : If you were to restate the proposition that prenatal malnutrition leads to a reduction in the number of brain cells and then put this in the form : at various stages what cell type centres of the brain are being influenced, and what cell types are being influenced postnatally by malnutrition. Perhaps it would be helpful in discussing performance.

M. WINICK : I don't know whether it would be helpful in discussing performance because that's not my area of competence, but I think the question you raise is a very interesting one, because what is being suggested in a way is : are we converting simply a quantitative change into what is, in fact, a qualitative change, according to the time that we impose the malnutrition ? For example, if we impose malnutrition at a specific time, and affect cell division of neurones in a particular area, because cell division of neurones is occurring at that time, are we left with a qualitatively different brain than if the malnutrition occurs at a different time when we are affecting glia in another area ? Now, as far as the human brain is concerned, we know very little about. Dobbing has recently published data which would indicate that there are two peaks of DNA synthesis in human brain. The first peak is occuring at about 20 weeks after conception and there is a second peak of DNA synthesis which is occurring at around birth. He has interpreted these data to mean that the major neuronal division is occurring early, at around 20 weeks, whereas the peak occurring later around birth, is mainly glial division, although this is inferential, there is no direct evidence that this is true. So that one may say that prenatal malnutrition may be more apt to affect neuronal division and that if you hit it at the peak of 20 weeks, and this is pure speculation on my part, it might be conceivable that you are hitting at the peak rate of neuronal cell division in the human. Now in the rat, the peak rates of cell division are quite different; as I showed you, there are two peaks in cerebellum, we think the first peak, again, relates to a different cell type, but we have no direct evidence for that. Now what this might mean relating to function, I really can't say, because I have no competence in the area of structure to function relation. I'm not a neurologist.

O. HECHTER : Can I ask another question ? Is there any evidence that what the psychologists call imprinting relates to changes in DNA and RNA in specific cell types ?

M. WINICK : Well, there are some data that would indicate that there is some relation between what the psychologists would call « imprinting » and

malnutrition during early life. These are data coming from Chow who has shown that if one malnourishes an animal from birth until weaning, this animal, even after he is totally rehabilitated, has an abnormal nitrogen metabolism in terms of the way he utilizes nitrogen. So that he will actually eat more and waste nitrogen in the form of an increased excretion of amino acids and other types of nitrogen in his urine, for the rest of his life, no matter how you re-feed him. So that in that sense, you have perhaps imprinted this kind of a nitrogen metabolism in this animal. I think, using this concept, that this might be the only example I can think of. Now in terms of whether or not you imprint psychological things, well, I just don't know what this imprinting really means. You certainly can produce permanent behavioural changes in animals. If this is what you mean by imprinting, it can be done. I just don't want to get involved in this area because I don't really know what imprinting means.

Journée du 2 décembre 1971

Deuxième séance

STRATÉGIE SCIENTIFIQUE

PRESIDENT JAMES R. MILLER

James R. MILLER
Epidemiological methods of detecting new birth defects

Hideo NISHIMURA
Embryological approach to human teratology

Discussions

EPIDEMIOLOGICAL METHODS OF DETECTING NEW BIRTH DEFECTS

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It is with a considerable degree of humility that I discuss this topic before such a distinguished audience in view of the fact that the effects of all of the proven teratogenic agents in man have been detected first by astute clinical observers and not by any elegant epidemiological methods. For reasons which are obvious, we shall always have to rely upon clinical observations in the detection of new anomalies but it is my contention that no one method is sufficiently adequate to serve as the sole method of detection and epidemiological methods involving the monitoring of large population groups must be implemented.

Criteria for Detecting new Patterns of Congenital Malformation.

Newcombe has set out three criteria which must be met by any program designed to detect new patterns of malformation :

1. A high degree of specifity in the diagnosis and classification of anomalies;
2. Early detection of anomalies;
3. Improved methods for the recording, rapid accumulation and communication of data on such anomalies.

1. *In addition to the high degree of specificity*, I should like to add accuracy, completeness and uniformity of diagnosis.

The need for accuracy, completeness and uniformity of diagnosis along with a high degree of specifity seems so self-evident, that it should not need any comment, but in practice it is so poorly managed that it must be mentioned. If detection programs were based on data gathered by a large number of specialists in various areas of congenital anomalies, each with its

own precise classification of specific defects, as we heve heard this morning from Dr. McBride, there would be no problem; but most surveillance schemes must depend upon large volumes of data based on the observations of many physicians, midwives and allied health personnel who have had no special experience with the problems associated with the detection of anomalies and birth defects. In many instances, the anomalies are of such a nature that anyone could detect and describe them, for example — the major surface defects such as cleft lip and palate, absence of tissues, (for example absence of ears, absence of limbs) and spina bifida and anencephaly. However, it may well be, and probably is, true that the critical problem is not a single major defect but an array pattern of defects, namely syndrome identification. This is an extremely complex area even for experienced clinical observers. I say this, because almost all (there are a few exceptions) of the known environmental teratogens in man produce not an increase in the frequency of common congenital malformations but a specific array of effects which we designate as the rubella syndrome and the thalidomide syndrome. This problem of the need for a high degree of specifity, accuracy and uniformity of diagnostic criterian is a most difficult one to guarantee and necessitates constant policing and education of collaborators in a surveillance project in order that each one clearly understands the importance of having precise uniform diagnosis and coding.

2. *Early detection.*

Teratogens act during the period of embryogenesis, that is during the first eight weeks of human life. Yet, the first observations on their impact are made thirty of more weeks later. If a new teratogen is not to be the cause of an incredible amount of human tragedy, it must be detected and, hopefully, eliminated as promptly as possible. In most instances then, reporting of anomalous infants should be made as soon after they are observed as possible, and an efficient surveillance program can not rely on longterm follow up to obtained information, although this most certainly will provide a better estimate of the true incident rate.

Although major surface defects should be detected and recorded at birth-but are not always-many internal anomalies such as congenital heart defect and many minor anomalies which may be important in syndrome identification will be missed (fig. 1). In order to overcome at least some of these difficulties, multiple sources of ascertainment should be used in any monitoring scheme. Although, the utilization of several sources of ascertainment may cause problems with respect to editing to ensure an unduplicated case load, these will be small in number compared with the advantages of using many sources such as physician's notice of birth, live and

stillbirth records, infant death records, and hospital records as sources of primary data. Where possible, it might be useful to use special statistics to supplement these estimates derived from the study of term infants. These might be derived from embryos and fetuses for example and I shall return to this point later.

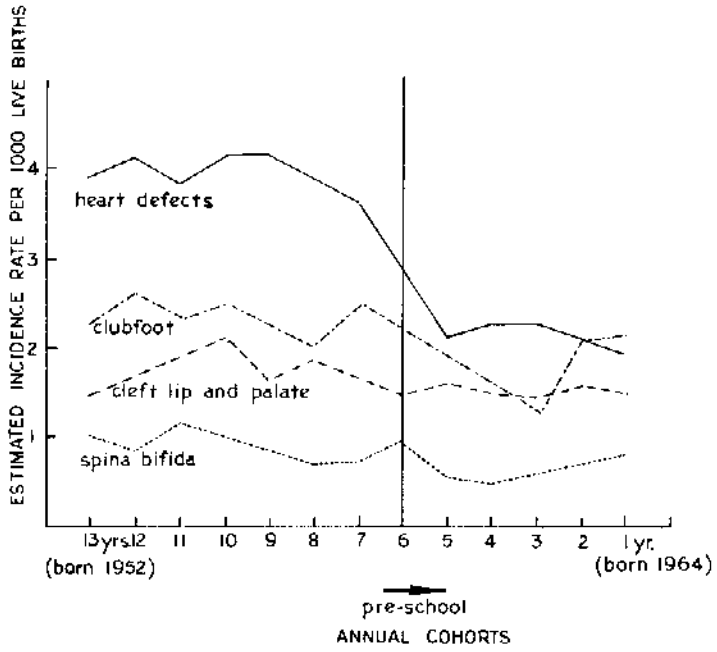

Fig. 1. Estimated incidence rates for four common congenital malformations based on data derived from the British Columbia Registry for Handicapped Children and Adults (from *Methods for Teratological Studies in Experimental Animals and Man* edited by H. Nishimura and J.R. Miller, Igaku Shoin Ltd., Tokyo).

3. *Improved methods for recording rapid accumulation and communication of such anomalies.*

The methods of recording accumulative and communicative results should be as efficient and as simple as possible, because the speed is of the essence. I personally do not believe that there is time to carry out sophisticated techniques to measure geographic or temporal clustering patterns as desirable as these may be in certain specific studies. What is absolutely essential, and probably can be accomplished by some simple card system or a tabulating system based on a computer, is a simple method of rapidly counting and tabulating types of anomalies and then providing statistics for the collaborating centres.

Statistical analysis.

In my opinion the most critical problem to be faced in any such epidemiological study is the need for reliable base line statistics. It is true that despite a great interest in the area of congenital malformations few societies can produce such statistics with respect to hospital, city, province, state or country, yet without this base line, it is very difficult to determine whether an upward swing is random or indicates over-zealous reporting or an indication of real concern. In other words, there is a difficult task in deciding the criteria to be used for sounding an alarm bell based on surveillance data without some clues as to types and of frequency of defects to be expected within normal limits. The question emerges as to how many years must a program operate just to collect reliable base line statistics? How much fluctuation around a theoretical or calculated mean should be tolerated?

Are annual analyses adequate or should quaterly statistics be used?

When new programs are introduced, one must be conscious of the fact that reporter bias could influence observed rates of a particular defect. For example, Milham in a surveillance system using defects recorded upon birth certificates in Washington and upper New York State found a spurious pseudo-epidemic of « miscellaneous genital-urinary anomalies » which could be traced to one physician in a country in upper N.Y. State, who reported all male newborns of having congenital phimosis, presumably, to justify circumcision. Educational programs attached to a surveillance system will give increased rates in specific defects which are simply a reflection of better reporting rather than indication of some new teratogenic agent in the environment. For example — Weatherall reports that the rates of congenital dislocation of the hip rose from 4.2 per 10,000 births in 1965 to 7.6 per 10,000 births in 1968 over a 3-year period in a study in Great Britain and this increase could be simply attributed to an educational program encouraging the reporting of specific congenital malformations.

Since we have no guidelines about how frequent statistics should be compiled, what comparison should be made, etc., it would seem that many different systems should be employed, at least till one or two are demonstrated to be sensitive enough to detect changes in incidence rates, which are considered to be reliable. For example — the calculation of estimated mean incidence rates w/appropriate error terms should be an interim process constantly changing as new data are added. Presumably, several different comparisons should be used to detect changes in rates. For example quaterly comparisons might be suitable for the detection of sharp changes in the frequency of anomalies but might be quite insensitive to subtle changes which gradually accumulate over a long period of time.

Although, the bulk of the data in any epidemiological program designed to detect new birth defects will come from studies of large populations of newborn infants, it is possible that other special studies of sub-groups might provide useful information and would merit attention. I should like to discuss two of these, first the potential use of sex and racial differences in the incidence of congenital defect and second, the potential value of embryos and fetuses.

It has been repeatedly demonstrated that striking sex (table 1) and racial (table 2) differences exist with respect to the frequency of occurrence of certain

TABLE 1

Number of cases registered with selected malformations, born 1952-1964 *and estimated sex-specific incidence rates per* 1,000 *live births. Data derived from British Columbia Registry for Handicapped Children and Adults. (from* Methods for Teratological Studies in Experimental Animals and Man, edited by H. Nishimura and J.R. Miller. Igaku Shoin Ltd., Tokyo).

Malformation	Number of cases		Rate per 1000 live births	
	male	female	male	female
Clubfoot	609	419	2·51	1·81
Congenital dislocation of hip	49	217	0·20	0·94
Spina bifida and meningocoele	187	214	0·77	0·93
Cleft lip with palate	233	138	0·96	0·60
Cleft lip (isolated)	123	54	0·51	0·23
Cleft palate (isolated)	125	128	0·52	0·55

TABLE 2

Rates for cleft lip and palate per 1,000 *births amongst North American Indians and non-Indians in British Columbia in* 1952-1964. (*from* R.B. Lowry, and D.H.G. Renwick, Journal of Medical Genetics, **6**, 67-69, 1969).

Type of cleft	Indian	Non-Indian	Total
Cleft lip only	0·25	0·38	0·37
Cleft palate only	0·44	0·54	0·54
Cleft lip and palate	2·48	0·71	0·78
All forms	3·17	1·63	1·69

congenital defects. The underlying reasons for these differences are not obvious in most instances but need not concern us here. However, in populations in which reliable base line data are available, it seems to me that the existence of such differences could be used effectively in monitoring programs. It seems to me that a significant departure from the expected sex ratio for any specific anomaly or a change in race specific incidence rates might be a sensitive indicator of the presence of a new teratogenic agent in the environment. It should be stressed however, that such procedures have potential use only in those situations in which good base line data are available.

At the present time, surveillance schemes are based on observations of live or still born (term) infants and as I indicated just a moment ago, this probably will be the case for a long time to come in most monitoring programs. However, since the great majority of developmental defects observed at term as " congenital malformations " occur within the first eight weeks of intra-uterine life, it would seem reasonable to suggest that there might be merit in incorporating the examination of aborted embryos and fetuses into a good surveillance program. Any consistent increase in the incidence of developmental defects in such abortuses would suggest that a new teratogene was operating and more importantly would result in a search for such an agent at least several months before its presence might be suspected from studies on term infants. In addition, since the time between the initiation of the action of a teratogen and the observation of the anomaly would be much shortened, it might be easier to detect potential teratogens.

Studies on abortuses in different parts of the work indicate that such specimens possess a high frequency of early developmental anomalies which are readily detected and described. In view of the large number of abortions, both spontaneous and induced, which occur continuously, it seems reasonable that the study of abortuses would be a useful supplement to existing sources of information in surveillance schemes, such as the physicians' notices and health records. An example of this use will be presented laters. My colleague Dr. Poland and I have argued elsewhere that although use might be made of both spontaneous and induced abortuses for the purposes of surveillance, spontaneous abortuses would be the more useful.

Examples of situations in which surveillance studies have detected increases in rates of defects and new arrays of defects.

In view of my statement at the outset of my presentation regarding the respective contribution to date of the astute clinical observer and the elegant

epidemiological survey and the rather formidable problems I posed for the development of a successful monitoring system, it might seem that the possibilities of developing anything reasonable are almost nil. However this is not so, and there are now several examples of monitoring systems which have detected apparently significant changes in the rates of certain anomalies. While no specific teratogenic agent has been detected by such methods, the fact that the systems despite their inadequacy were sensitive enough to detect a significant change, is encouraging.

1. Leck & Smithells have reported that a program involving routine reporting of congenital malformations in Liverpool prior to the Thalidomide episode was sensitive enough to pick up an increase in limb reduction anomalies in August of 1960, although something was suspected at that time, the report on this observation did not reach the literature until two years later, long after the reports of McBride and Lenz had implicated Thalidomide in the production of such reduction anomalies. I believe this is a good example of the need for the rapid feed-back of results of surveillance programs which was I mentioned previously.

2. Within the last few years, Kallen & Windberg have reported on the development of a Swedish register of congenital malformations covering about 80,000 births annually. Although this registry was not in existence at the time, Kallen and Winberg have used the method in existence at present to test available Swedish data from the time of the Thalidomide episode. It is their contention the results indicate that had the register been operating at the time, it would have identified an epidemic a few months after the appearance of the first Thalidomide embryopathy. In addition, Kallen and Winberg have approached the problem of syndrome identification by a detailed analysis on 120 children born with multiple malformations reported to the Swedish registrar. On the basis of these analysis, they felt they had detected some " new syndrome " (1) which consisted of closure defect of the central nervous system and anophtalmia and microphtalmia, cleft lip and/or palate and reduction malformation of the limbs, not related to the amelia-phocomelia group. Children with this array of anomalies were common during the first half of 1965. Although, the authors could not relate this to any specific agent in the environment at that time, they point out that the system they used was sensitive enough to detect this particular event.

(1) A similar surveillance system based on defects recorded on birth certificates in upstate New York has presented evidence that it may have been sensitive (in retrospect) to detect a Rubella epidemic in 1964-65 and the tail of a mini-epidemic of thalidomide embryopathy in 1962.

3. I should like to talk about a Canadian study, which has been in existence for several years. A pilot study of a surveillance system of congenital malformations has been operating in Canada since 1966; the design of this program and its method of operation have been described by Dr. Philip Bannister on several occasions. Four provinces widely separated in the country — New Brunswick, Manitoba, Alberta and British Columbia forward information to a Central office in Ottawa, where the data are compiled and analysed. The total number of births annually is about 95 000. The information is based on infants born with congenital malformations and is obtained from the physicians' notice of birth form and death certification. By the end of June 1969, this surveillance system indicated what appeared to be an increase in the number of infants born with reduction deformities of the limbs, (defined as absence of bone or cartilage and/or absence of digit, hand, foot, forearm, arm or thigh). Since there did not appear to be a change in the incidence rates of other anomalies, a special effort was put into a program to pursue this observation on reduction anomalies. Local follow-up studies were initiated on all cases in an effort to determine the existence of any common teratogenic factor. In Alberta where the greatest number of cases occured (table 3) this was a very elaborate and detailed study while in the other provinces, a less concentrated effort was carried out. Detailed studies yielded no specific clues as to an etiological agent. As the data in the table indicate, this increase in the incidence rate persisted in Alberta into 1970

TABLE 3

Number of cases and rates per 100,000 *live births with reduction deformities of the limbs in four Canadian provinces by year,* 1966-1970. *(Data courtesy of Dr. Philip Banister).*

	1966		1967		1968		1969		1970	
	cases	rate*	cases	rate*	cases	rate*	cases	rate*	cases	rate*
New Brunswick	4	31.4	5	40.5	4	34.5	6	50.6	7	60.5
Manitoba	3	16.7	6	34.9	11	63.1	5	27.4	7	37.8
Alberta	10	32.7	7	22.8	17	56.4	20	64.8	25	78.3
British Columbia	13	40.0	15	45.6	15	44.5	16	45.1	31	84.5
Total	30	32.0	33	35.4	47	50.6	47	48.7	70	70.9

* per 100,000 live births

and the same intensive follow-up of cases carried out in Alberta was initiated in rBitish Columbia in 1970. Again these results did not yield any clues regarding possible responsible factors. The evidence in 1971 to date, is that the incidence rate for this particular defects has now dropped, back to normal levels.

Therefore, despite the inability of the follow-up studies which were initiated quickly to detect a specific etiological agent, this example has proven again at least to my mind that the surveillance system even one in a country as large as Canada and as diverse, is capable of demonstrating changes in the incidence of anomalies and in properly initiating follow-ups.

4. *Embryo and Fetus study*. At the time the pilot surveillance study, which I have just described first detected an increase in the incidence rate of limb anomalies, Dr. Poland and I decided to re-examine the frequency of these defects in a series of human embryos and fetuses which we have been collecting since 1965, in order to supplement statistics from B.C. on live and still births which were already part of the surveillance program. The data *in the table* 4 presents our findings. It can be seen that there was a sharp increase in the occurrence of reduction deformities which was statistically significant at the 0.05 level during the 68-69 period. There was no corresponding increase in spinal rachischesis and no overall increase in the incidence of all types of anomalies specially during this period.

TABLE 4

Relation of number of specimens of human embryos and fetuses with limb reduction deformities and spinal rachischisis to total number of specimens and anomalous specimens (*from* J.R. Miller, and B.J. Poland, *C.M.A.J.*, **103**, 503-505, 1970).

Year	Total spontaneous abortions	Total abnormal specimens	Reduction deformities			Spinal rachischisis		
			No.	% of total	% of abnormal specimens	No.	% of total	% of abnormal specimens
1965-66	95	43	3	3	7	4	4	9
1966-67	115	47	3	3	6	5	4	10
1967-68	140	63	0	0	0	10	5	16
1968-69	148	70	9	6	13	10	6	14

Although, it is not shown in this table, the number of specimens with limb reduction defects dropped back to the previous level in '69-70 and we predicted on this basis that the rate observed in newborns would drop in '71 and a preliminary evidence indicates that this has happened as I mentioned previously.

A review of the case records of the women from whom the specimens with limb reductions were obtained failed to detect a common feature which might provide an etiological clue. However, we believe the results of these studies support our thesis, namely, that information obtained from the systematic study of embryos and fetuses can be used as an important supplementary data in monitoring systems.

Conclusion.

I have not mentioned a cost for such a program because data on this are not available to my knowledge. The only estimates of which I am aware are those calculated by Bannister for the Canadian Study. A year ago, the estimated cost of monitoring each birth insert is about 0.35 dollars and the cost of detecting an anomaly is $ 5.78 and the cost of each case recorded is $ 8.18. This appears to be expensive programme and of course, does not cover the costs of special follow-up studies which are initiated when an apparent increase in the frequency of a specific defect or array of defects is annuonced; but these costs should be lowered with increased automation.

Perhaps, skeptics would say that the case for epidemiological techniques in the detection of new birth defects or array of birth defects remains " not proven " but I cannot agree. While acknowledging the formidable challenge in developing such schemes, I do not believe we can afford not to implement some program in view of the incredible cost of a potential disaster and in view of the still poorly defined regulations governing the introduction of new chemicals, drugs, in most countries. While I still have great faith in the powers of the astute clinical observer, I do not believe we can rely upon this as our sole means of monitoring populations for the presence of new teratogenic agents.

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EMBRYOLOGICAL APPROACH TO HUMAN TERATOLOGY

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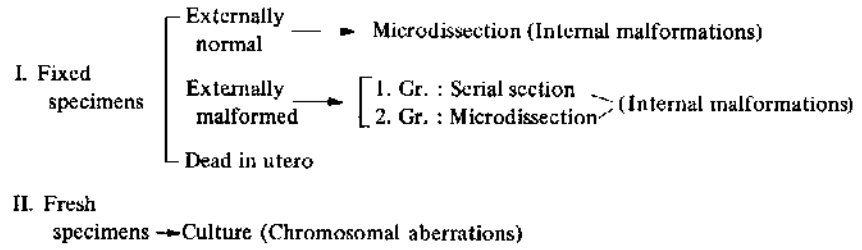
For the past ten years, we have continued a systematic survey of defective embryos among early intra-uterine population. This study has been made under the collaboration of a large-scale team of obstetricians, and is unique in terms of the facts that the specimens were obtained by induced abortion, that is, arose from healthy pregnancy and the population examined is judged to preserve the embryos with initiating malformations which will be lost selectively during the later course of pregnancy. So far, we have obtained about 30,000 embryonic specimens. Approximately 6,000 undamaged embryos among them were used as the main materials for the present study. All of those were accompanied by a case history record. The developmental stage of the specimens ranges from Streeter's horizon nine through 23, namely three to eight weeks of fertilization age. The procedure of examination of the fixed specimens was external observation followed by serial sections of the whole embryos or microdissection. About 600 fresh specimens were subjected to cytogenetic study by culture method (shown as follows).

Systematic survey for anomalies in human embryos of 3 to 7 fertilization weeks



Now, what would be characteristic features of the defected malformations ? The following four points can be pointed out :

1. Certain severe anomalies were found far frequently compared with the prevalence in newborns.

For example, the prevalence of holoprosencephaly at 3.2 % is 40 times higher than that in newborns [3]. High incidence of other central nervous system anomalies were also indicated : the frequencies of exencephaly and myeloschisis were as high as 2.8 % and 3.0 % respectively [1]. Among various cardiac anomalies, persistent atrioventricular canal which is one of the severest types was found in the frequency of 8.0 %, while this anomaly has been very rarely seen in infants. Most of such cases with severe malformations are probably imcompatible with live birth. This means that such materials are exceptionally useful for study of the preceding process of intra-uterine death.

TABLE 1

Chromosomal aberrations in embryos of 3 to 7 fertilization weeks (Tonomura's group).

No. of cases karyotyped	Cases with chromosomal abberation	
	No., ( ) : %	Type, ( ) : no.
563	12 (2.1)	45, X (3) 45, XX, D-, G-, t(DqGq) + (1) 47, XXY (1) 47, XX, E + (1) 47, XX, G + (2) 47, XY, G(1) 47, XY, D + (1) 46, XY, ace (1) 69, XXY, (1)

Next, the cytogenetic study was conducted on 563 embryos alive *in utero* by Tonomura's group. The frequency of chromosomal aberrations was found to be 2.1 %, that is about three times higher than that in live births (Table 1).

2. A variety of initiating figures of malformations was demonstrated.

For example, it was shown that, in addition to non-closure of the neural tube, rupture of the closed neural tube is one of the pathways of formation of myeloschisis. As another example, I will take up the subcutaneous bleb in the nape which occurs at about 7 % of the embryos. It was known histologically that this is the result of abnormal accumulation of cerebrospinal fluid discharged through the ruptured roof of the hind brain. It may be presumed that this is the initiating picture of meningocele. Underdevelopment of the mandibular arch is also a characteristic anomaly. This is estimated to present the early stage of micrognathia. The next example is the polydactyly which was found most frequently among limb defects. Observation of a closed developmental series of this defect revealed an interesting finding that disappearance of the apical ectodermal ridge, a thick skin ectoderm which appears only during earlier stage, is retarded on the surface of the extradigit of the preaxial polydactyly, while this is not the case with postaxial polydactyly.

Thus, our collection of defective embryos can demonstrate actual pictures of teratogenesis in man. Furthermore, that will allow us to compare the pathogenesis of various defects between animals and man, and will contribute in evaluation of animal studies.

3. Thourough study of serial sections of the whole embryos with external malformations indicated that about 40 % of these specimens were accompanied by some other internal malformations in various organ systems (Table 2).

TABLE 2

Visceral defects combined with various external malformations in human embryos.

Embryo No.	Horizon	CR Length (mm)	Sex	External malformations	Combined internal anomalies	Remarks
4972	20	18.5	?	exencephaly, myeloschisis	double vertebral body and notochord, complicated atresai in digestive tract, hypoplasia of gonads	hyperthyroidism
21973	17	9.8		exencephaly, holoprosencephaly, myeloschisis	———	common cold, vaccine for flue, diginin
1208	17	11,8		holoprosencephaly, polydactyly (r-hand)	blood stasis in heart, liver and mesonephros*	spontaneous abortion
10049	16	10.3		holoprosencephaly, polydactyly (l-hand)	blood stasis in scalp, heart and mesonephros*	genital bleeding
4540	18	13.9	♀	holoprosencephaly	———	
565	17	11,1		holoprosencephaly, myeloschisis	———	
19551	17	13.0		holoprosencephaly	———	genital bleeding, cleft palate in brother
7182	19	11,1	♀	holoprosencephaly	hypoplasia of adrenal certex	threatened abortion
10341	16	7.3		holoprosencephaly	———	
14742	17	8.2		holoprosencephaly, polydactyly (r-, l-hands)	focal vesicle in forebrain	epilepsy
21821	17			holoprosencephaly, polydactyly (r-hand)	———	
9953	18	10.8	♂	holoprosencephaly, polydactyly (r-, l-hands)	———	38 yr, genital bleeding
8791	16	8.3		holoprosencephaly	———	
391	21	20.0	♀	embryonic hydrocephaly	———	
3191	21	21,7	♂	cleft lip (r), split hand (l)	VSD	
9205	19	17,3	♀	cleft lip (r)	blood stasis in scalp, mandiable, late rathorax*	
17356	17	12.1		cleft lip (r, l)	———	
17984	17	12.7		cleft lip (r, l)	fused metanephros	35 yr
719	14			myeloschisis	hypoplasia of mesonephros, blood stasis in liver, mesonephros*	
18289	20	18.0	♀	myeloschisis	———	35 yr, myoma uteri
888	18	11.1	♀	polydactyly (l-hand)	hypoplasia of thyroid, fused metanephros	
8003	23	23,9	♀	polydactyly (l-hand)	annular pancreas	common cold
1865	21	22.2	♀	polydactyly (l-hand)	esophageal atresia, esophagotracheal fistula, VSD, SUA	anemia

* Signs indicating threatened death in utero ?

Our goal of the further study along this line is to know some of the successive chains of teratogenesis in man.

4. Some minor anomalous formations which appear to exist only transitionally, were occasionally found.

For example, one or two small tubercles were occasionally found on the digit, tail or on the basal part of hyoid arch of five-week-embryos. Also, it was peculiar that the internal cardiac wall of six and a half-week-embryos showed a small protrusion near the pulmonary valves in the frequency of about 20 %.

Such formations mentioned above have never been described in the recent literature [2] and their meaning remains to be clarified.

Next, I will mention about our correlation study aimed at identification of certain internal factors associated with occurrence of the defects or death of embryos. Significance of such a retrospective study may be justified by a specificity of the examined population *in utero* and the advantage that patients' memory is comparatively accurate. Although the number of the malformed cases, near 100 in total, is still small, the preliminary analysis was done with respect to 30 items in the case history records. A significant positive association was shown between embryonic malformations and each of maternal genital bleeding

TABLE 3

Maternal genital bleeding and pathological embryos

Genital bleeding	Total no.	No. of malform. ( ) : %	No. of wastage ( ) : %
-	2771	44 (1.6)	120 (4.3)
+	395	13 (*3.6**)	51 (*14.2***)
++#	390	24 (*6.2***)	169 (*43.4***)

Accompanied by symptoms of threatened abortion.
* $P < 0.05$ ** $P < 0.01$.

TABLE 4

Hyperthyroidism during pregnancy and pathological embryos.

Hyper-thyroidism	Total no.	No. of malf. ( ) : %	No. of death *in utero* ( ) : %
-	5160	59 (1.1)	826 (16.0)
	11	2 (18.2)**	1 (9.1)

** $P < 0.01$.

TABLE 5

Radiation during pregnancy and pathological embryos.

Radiation	Total no.	No. of malf. ( ) : %	No. of death *in utero* ( ) : %
-	5038	63 (1.25)	783 (15.54)
+	96	3 (3.13)	23 (23.96)*

* $P < 0.05$.

TABLE 6

History of uptake of drugs for anemia and pathological embryos.

Drug	Total no.	No. of malf. ( ) : %	No. of death *in utero* ( ) : %
-	5083	66 (1.30)	802 (15.78)
+	19	0	8 (42.11)**

** $P < 0.01$.

TABLE 7

History of antibiotics uptake and pathological embryos.

Antibiotics uptake	Total no.	No. of malf. ( ) : %	No. of death *in utero* ( ) : %
-	5042	66 (1.3)	788 (15.6)
+	60	0	22 (36.7)**

** $P < 0.01$.

TABLE 8

Uptake of vitamins (excluding vitamin K) and pathological embryos.

Vitamins uptake	Total no.	No. of malf. ( ) : %	No. of death *in utero* ( ) : %
-	4942	64 (1.3)	767 (15.5)
+	160	2 (1.3)	43 (26.9)**

** $P < 0.01$.

during the terminated pregnancy and maternal hyperthyroidism, between embryonic death and each of maternal irradiation and intake of drugs for anemia, antibiotics or vitamins (Tables 3-8).

Concludingly, our embryological studies to be continued further will be able to meet the needs not realized by any other studies.

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DISCUSSIONS

J. MILLER : Thank you very much, Professor Nishimura. Dr. McBride, would you like to make some comments ? Before we turn the discussion to the audience ?

W. MCBRIDE : I would be delighted to comment. I enjoyed Professor Miller's paper immensely.

I dont't think he has to justifiy the cost of these studies. I feel that the cost of a child with severe physical or mental retardation to the community is much greater than the cost of his surveillance methods. This child, if born, will have a life expectancy approximately the same as any other member of the community, his ability to work may be impaired, and he may, perhaps, be supported by the community, for the rest of his life. So I feel that the cost doesn't have to be justified.

As regards Professor Nishimura, I regret that Japan, not quite as bad as Australia, but is a little bit off the beaten track — even from Europe, I think it is about a 14-hour flight — If one has never visited the city of Kyoto which is the most beautiful city, in Japan, it is worth going there to see the Nishimura collection, if you're interested in congenital anomalies. I hope that one day the World Health Organisation in its wisdom, may find a home for this collection, perhaps in Europe or perhaps in New York, somewhere where it will be within easy access for students of this subject because it is the most amazing collection I have ever seen.

I was interested to see, on one of Professor Nishimura's slides, the incidence of congenital anomalies increased where the mothers had taken or been given treatment for anaemia — *drugs for anaemia.* I hope he might comment on this later. I feel that our main hope for the prevention of congenital defects at present lies in the identification of environmental factors which permit or encourage abnormal development, and in their elimination or correction. I think the concept of optimal maternal health must be examined, analysed and achieved. I think Dr. Whitehead this morning emphasised this point too. I think that we have certain indications for increased vigilance. We should be on the alert if the mother is of increasing age : increasing age and parity, I think, are factors which we must consider and be on the alert for congenital anomalies. Secondly, family history of anomalies. Thirdly, the history of recurrent abortions in the mother. Fourthly, maternal ill-health, particularly in the first trimester of pregnancy. We should note what virus diseases, even the common cold or the Echo virus, or

what drugs she has ingested in the first trimester of pregnancy. Fifthly, I think chronic maternal illnesses, such as diabetes. Sixthly, exposure to known teratogens, irradiation, although care is taken these days to shield the fetus. The seventh point is the amount of amniotic fluid which gives us some clue as to the welfare of the fetus. The eighth point is persistent fetal malpresentation or abnormal attitude, and I think these should be noted in every pregnancy and recorded at the time of birth in a detailed history, which, I hope, will be put on to a computer.

I think that these two papers this afternoon, I call Professor Nishimura's comments a paper because of those beautiful slides which he showed, and the immense study which he has done, and I feel that our hope, for the future — I am not playing down the genetic side of anomalies, — are an intense amount of study of environmental factors.

Thank you.

J. MILLER : I neglected in my presentation to talk about the situation in which there might be a rather large population exposed to a potential teratogen. It seems to me that some group, (government, Who, etc.) should be prepared to send in a team to investigate such a situation very quickly. The point has been made that we should perhaps pick out special groups for immediate study rather than wait for such potential disaster. I am not as keen an advocate for prospective studies as Professor Degenhardt and others were this morning. I don't believe such studies have ever been rewarding and I don't think they ever will be, because they begin too late. We must begin looking for agents before the woman is pregnant (if this evidence presented this afternoon is valid) and I don't believe any studies can reasonably begin this early. To date prospective studies can reasonably begin this early. To date prospective studies begin when women first come to a clinic for their first prenatal visit and that's too late because they usually come after the critical period of embryogenesis. So unless there is some program established that has mammoth support and attempts to establish a large cohort of women who begin filling in " diaries " before they are pregnant, and continue to record all relevant information throughout the period of fertilization and early embryogenesis, I just don't see where prospective studies will yield relevant information.

The discussion is now open, for any member of the audience who wishes to participate, either to ask questions or to comment upon this problem of the detection of anomalies : when they can be detected, and what we can do about it.

C. LEVINTHAL : In view of the very large effect of smoking on disease in adults — much higher than anything associated with urban environmental pollution —

it would seem to be important to look closely at correlation between defects and smoking. Can one put some light on the teratogenic effect of human smoking ?

H. NISHIMURA : There is an item on smoking plus or minus and a preliminary analysis was made between maternal smoking and the occurrence of defective embryos or deaths of embryos. However, there is no association, no significant association has been found as yet.

H. LEWIS : Are there any data correlating coffee consumption (caffein intake) with developmental abnormalities ?

J. MILLER : Not to my knowledge. But again, it is the problem of dealing with a very common agent which it would be most difficult to implicate.

W. MCBRIDE : Methotrexate has been used in the treatment of psoriasis by dermatologist. Some of the women were pregnant or became pregnant whilst on this treatment, some have given birth to babies with anencephaly or microcephaly. This emphasises the importance that *if* it is *necessary* to give women of child bearing age a known teratogen, the woman and her medical supervisors must be aware of the dangers and must make certain that she does not become pregnant. It should be considered essential that such women would take oral contraceptives hormonal preparation, or be given intramuscular long acting hormonal preparations, to be certain that she does not become pregnant whilst on treatment or even for there months after completion of the methotrexate therapy.

S. BENNETT : I have two questions for Professor Nishimura. With respect to the series of embryos which you have studied so ably, what proportion were from spontaneous abortions and what from surgically induced abortions ? Second, of those removed surgically, what methods of removal were used to obtain such a significant proportion with little physical damage to the specimen ?

H. NISHIMURA : To your first question : the materials dealt with in this paper is concerned with only induced abortion. Although we have been obtaining specimens from threatened abortions and spontaneous abortions, these were not included to my data shown to you at all. To your second question : the method of the operation is in most cases cervical dilatation and currettage. This is the most common method for interruption of pregnancy at the second to third month.

Sometimes, hysterectomy has been conducted. But I must add that nowadays the method of suction is gradually becoming popular. By the last method,

it is very difficult to get good specimens. So, as far as the anatomical studies are concerned, the method of curettage is most favourable.

J. MILLER : If there are no further questions, may I go back to some points which were raised earlier ? I don't like to discuss questions about costs, but nevertheless, people who are questioning whether such surveillance systems play a role in public health services, do ask such questions. One of my points against prospective studies is in terms of actual practicality, that is convincing public health authorities about such schemes and their importance, etc. It would be very difficult to justify studies of the required magnitude on a cost basis. However, it seems to me that the use of embryos and fetuses is a happy compromise because it is possible to get back to a developmental stage which is very close to the initiating events when the mother's and physician's memory about particular situations are clearer. So Professor Nishimura's study, while still a retrospective one, is much closer to the initiating events and may answer some of the questions which prospective studies are designed to investigate. It seems to me therefore that it would be easier to justify the costs of a study involving embryos and fetuses than of a large prospective survey.

Journée du 2 décembre 1971

Troisième séance

STRATÉGIE SCIENTIFIQUE

PRÉSIDENT JAMES R. MILLER

T. H. INGALLS and M. YAMAMOTO

Creation of chromosomal aneuploidies and polyploidies in the animal embryo

Discussions

CREATION OF CHROMOSOMAL ANEUPLOIDIES AND POLYPLOIDIES IN THE ANIMAL EMBRYO

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Ours was really a study in experimental epidemiology in that hazy area which Dr. Miller himself just brought up — the border between cytogenetics and teratology. I suppose the area where Dr. Yamamoto and I have been working the last four or five months is wholly in cytogenetics. Let me first give the background, which starts out more than twenty years ago. About 1949, together with two of my colleagues, Prindle and Curley, I undertook to test the teratogenic properties of hypoxic stresses of pregnant mice treated in a low pressure chamber during the latter half of pregnancy. The whole area of acquired congenital defects in human beings had just been opened up, as Dr. McBride noted this morning by Gregg's wartime discovery of the rubella syndrome in Australia. Moreover, the promise of experimental methods for such exploration in animals had been demonstrated by Warkany and Nelson's study of the effect of riboflavin deficient diets in pregnant rats. Our own interest in the use of hypoxia stemmed from a study of the events of early pregnancy of mothers who had given birth to mongoloid babies at term. It seemed important to take the whole problem of congenital deformity in relation to disturbances of pregnancy into the laboratory for analysis, using a small animal model such as the mouse, and a naturally occurring teratogenic agent such as hypoxia.

The rationale of using hypoxia had to do with haemorrhage, and contusion and hemostagnation; there were several reasons including automobile accidents and abdominal trauma followed by mongolism.

It is hardly necessary to add before this audience that the whole horizon was to be expanded shortly by Lejeune's demonstration of mongoloid trisomy and the observations of thalidomide embryopathies by McBride and Lenz. Nonetheless, during the decade 1950-1960 it became clear from our own studies, from Degenhardt's work in rabbits, Wertheimer's work on hypoxia in the production of ocular defects, and others, that atmospheres equivalent to high

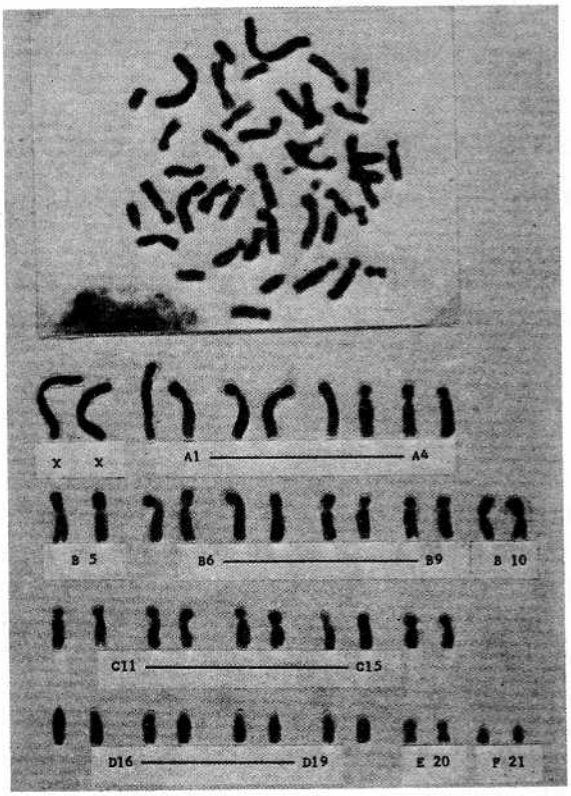

Fig. 1. Metaphase plate and karyotype of the normal female hamster.

altitudes of the order of 30,000 feet were indeed teratogenic for the developing embryo. The resulting malformations after such experiments could be related to the timing and the degree of maternal hypoxia and to the stage of gestation at which the hypoxic disturbance occurred. Additions to an enlarging understanding of the teratologic mechanism came from Grabowski at the University of Miami in Florida, and he made many physiologic studies of the subject over a 15-year period in the fifties and sixties. Also, the earlier studies that had been made of the role hyperbaric oxygen in causing retrolental fibroplasia and other studies from the field of aviation medicine made during World War II were relevant to our thinking.

All these works combined to explain the pathologic sequences resulting from hypoxia as due to an upset homeostasis characterized by lowered pH and carbonic and lactic acide accumulations.

Not only was the advent of cytogenetics as a scientific discipline necessary for the understanding of teratology to enlarge, but it has taken my Japenese

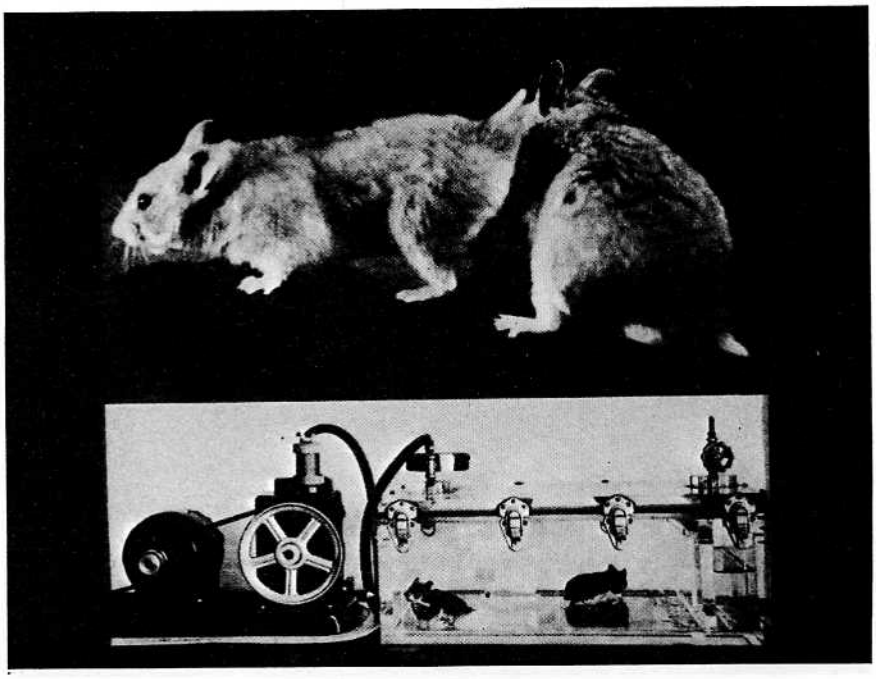

Fig. 2. *Top:* Female receptiveness. *Bottom:* Hypoxic stress of two female hamsters motionless within a low-pressure chamber.

co-workers and I another 15 years to appraise and compare the advantages and the disadvantages of Zebra fish, mice, rats, rabbits, sheep and finally hamsters in our search for the most suitable animal model for today's cytogenetic experimentation. We have come to rest upon the hamster as having a whole set of advantages over other small mammalian competitors; it is small, tame, inexpensive to feed and house and the females' estrous cycles come 4 days apart. Within 2 weeks we can reverse her circadian rhythms and this makes it possible to schedule reproductive experiments during the working day rather than at night. Under such circumstances, ovulation occurs early in the afternoon and copulation is readily effected at the start of a working day. Of additional importance to those planning cytogenetic experimentation, is the fact that hamster chromosomes are polymorphic and can be readily separated simply by inspection into 7 main groups that include a distinctive pair of X chromosomes (Fig. 1). Moreover, Dr. Yamamoto and I have found that, as with gross congenital defects, a constellation of chromosomal anomalies can be induced at the same negative pressures in the same plastic chamber (Fig. 2) so successfully used for many years to deliver timed teratogenic stresses later in pregnancy. These were maternal disturbances resulting in cranioschisis, spina bifida, cleft palate, bony

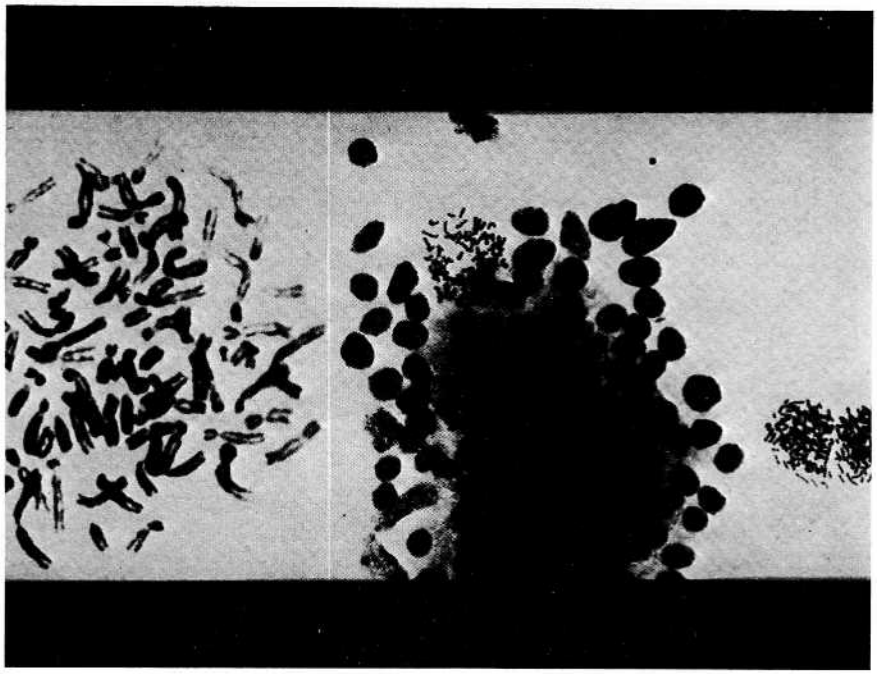

Fig. 3. *Left:* Three tetraploid plates in a 10 day old fetus. Maternal hypoxia for six hours at 30,000 feet three to nine hours postcoitus. *Right:* Tetraploid cell by oil immersion. After hypoxia for six hours at 30,000 feet three to nine hours postcoitus.

defects and vascular malformations of litter members. The same dosages turned out to be mutagenic just as they had been shown to be teratogenic. Dr. Yamamoto will shortly tell you of our first experiment made during the spring of 1971.

Following maternal hypoxia on the first day of pregnancy we have encountered mosaic tetraploidy (Fig. 3) when the stress was given not long after the time of fertilization and first cleavage, Triploidy (Fig. 4) and mosaic triploidy when the process of extruding the second polar body was interrupted around or just after the time of fertilization. We feel sure that a whole system of chromosomal anomalies has been uncovered which have their origins at one or more stages critical for normal reproduction : these include the first meiotic reduction division in the ovaries; the second meiotic division and the migration of the oocyte through the oviduct; the time or around the time of fertilization and initial cleavage; and finally the periods after fertilization and initial cleavages before the embryo « hatches » from the zona pellucida. We are not prepared today to document and substantiate more than one phase of this work, the action of

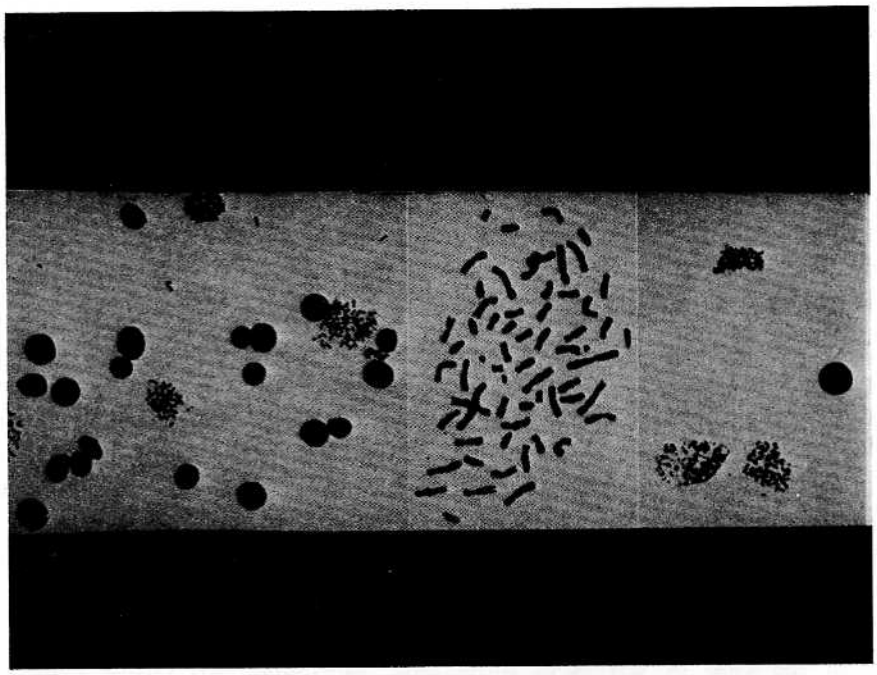

Fig. 4. Triploid sets of chromosomes. Mother exposed to hypoxia for six hours at 30,000 feet on the first day of pregnancy. *Top :* Four-cell embryo; *Center :* Metaphase spread; *Bottom :* 10-day old fetus.

hypoxia as a chromosomal mutagen and Dr. Yamamoto will tell you of work recently completed and readied for publication.

Such studies can not be applied unreserverdly to the interpretation of the polyploid and aneuploid formations observed in human beings. They can, however, be made, we believe, to serve as useful and indispensible guidelines to needed clinical studies of maternal-child relationships in human beings, particularly of children born after disturbances of meiosis, fertilization, or of very early pregnancy. Such pregnancies are often associated with placental and chromosomal anomalies — trisomies, monosomies, triploidies, tetraploidies — of the kind we can observe in the hamster embryo.

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I would like to report on chromosomal anomalies induced by hypoxic stresses given in a low pressure chamber during the very early hours of fertilization and pregnancy. In these experiments we selected golden hamsters for several reasons, such as their distinctive chromosome patterns, their 4 day

estrous cycle, and the ease of timed matings under the controlled lighting system described by Dr. Ingalls. In order to obtain the desired timing of copulation, we check for estrous discharge and mating behavior of the female when taken to the male's cage. When she is in estrus, she exhibits a lordotic posture, a sign that copulation will take place.

After the matings are completed, females are placed in the low pressure chamber for 3 hours or more around the estimated time of fertilization and first cleavage. The low pressure atmosphere used in our experiments usually corresponds to altitudes of 30,000 feet or above.

We analyzed chromosomes at both preimplantation and organogenetic stages. There were about 300 embryos harvested at preimplantation stages. Positive findings included a high percentage of triploid and tetraploid formations in the test groups, as contrasted to the controls. The same heteraploid patterns were observed also in fetuses sacrificed 9 days after copulation at organogenetic stages of development. According to the karyotypic analysis of triploid metaphase plates, sex chromosome complexes were all XXX or XXY patterns. Therefore, as an explanation of the origin of these triploid formations, failure of second polar body formation seems a likely cause of the anomaly.

In the case of tetraploid formations, we found two different mosaic patterns. One consists of higher concentrations of diploid-tetraploid mosaics (about 25 % to 50 % proportions of tetraploid cells). The other consists of lower concentrations of mosaics (less than 10 %). The differences are related to the different timings of hypoxia. We interpret the facts to indicate that the origin of these chromosomal mutations took place during the very early stages of preimplantation development and that the higher ratios were determined in cell clusters of 2, 3 and 4 cell stages of embryonic development, whereas the lower ratios became established a day or two later when the embryonic cluster consists of 8 to 16 cells.

Interestingly enough, we found three cases of endoreduplication at preimplantation stages. We found three cases of endoreduplication may represent one step in the genesis of tetraploid cell lines related to hypoxia.

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DISCUSSIONS

S. BENNETT : I am impressed by the studies of Professor Ingalls and Dr. Yamamoto relating to the critical importance of the timing of episodes of anoxia in producing fetal chromosome anomalies. An important environmental cause of episodic anoxia in humans is carbon monoxide. Can Professor Ingalls tell us what is known about the metabolic behaviour, or partition, of carbon monoxide between mother and fetus. Further, does he know of studies of the teratogenic effects of carbon monoxide.

T. INGALLS : I don't know of any direct physiological knwledge except that which relates smoking to carbon monoxide accumulation. However, a certain amount of clinical knowledge is available about carbon monoxide poisoning, followed by very serious congenital defects; for example, in the child of a mother who tried to commit suicide. There is one bit of experimentation that does seem related to our unpublished experiments indicating that delayed fertilization comes into the picture. For example, Dr. Yamamoto could produce various chromosomal aneuploidies, polyploidies and mosaics just by delaying fertilization in the hamster. The question arises, what about combinations of delayed fertilization and carbon monoxide poisoning or, let us say, carbon monoxide accumulations from smoking. There are many other ways also of producing relative hypoxia — from asthma, from emphysema and as far as the embryo in the uterus is concerned, from venous congestion, and from maternal decompression within an airplane. Also, when you get a uterine tube all scarred with adhesions, I think you get into another kind of delayed fertilization, so that I think there is a lot to be done in this whole area.

S. BENNETT : Final remark; may I suggest then, that there may be useful knowledge to be gained from studies of the behaviour and effect of carbon monoxide on the fetus and on pregnant animals. Moreover, I venture to suggest that it might be worthwhile to insert a search for carbon monoxide exposure in epidemiological studies such as those undertaken by Professors Miller and McBride.

T. INGALLS : I think you come back into an area of multiple causation almost too difficult to appraise — smoking, inhaling, number smoked, number of drinks had, whether a pregnant woman is taking the airplane overseas or not, pressurization of the cabin, etc. You come up against a good many factors that

could work together in multiple causation which we can't do much more than just mention.

W. McBRIDE : An interesting area that Professor Ingalls might consider studying is Africa, where hookworm and malaria are still prevalent, and you see women with very low haemoglobin levels, 3, 4, 5, grams, and in these women — the foetus must surely suffer from hypoxia right throughout pregnancy.

A. MONROY : Have you made chromosome analysis in the different regions of the embryo ?

T. INGALLS : No. Dr. Yamamoto and I have had several discussions about the possibility of taking an embryonic arm, or perhaps the head as a standard sample; I don't think he has ever been able to solve the technical problems involved but we would both agree with you; we should have done it.

M. YAMAMOTO : I cut the specimens into ten or fifteen pieces for direct chromosomal analysis. However, even in 9-day old fetuses it is impossible to investigate chromosomes from a specific part or tissue of the fetus.

A. E. HELLEGERS : I think there are a fair number of data known in this area which have perhaps not been mentioned. The first are studies of pregnancy at high altitude. At levels of 15 000 Feet in Morococha, Peru, Donald Barron and his coworkers were unable to find any particular problems of this kind. Now we did not go to Peru to do this particular kind of study, but rather to study oxygenation of the fetus *in utero*. However, perhaps a first rapid source of data would be available by screening a population like that of Morococha, With the exceedingly simple pregnancy tests of today one could from the known pregnancies proceed to collect the material and do the chromosomal analyses.

Perhaps a second pertinent point is that to the best of my knowledge human beings don't reproduce at altitudes much above 17 000 feet. It may very well be that nature has taken care of this problem particularly if your results refer to 30 000 feet.

A third pertinent point is that Dr. Neill and her coworkers have been in the process of studying mothers with Tetralogy of Fallot who had Blalock-Tanssig procedures performed on them and became pregnant. Where the oxygen saturations were much below 75 % on the hematocrits over 65 (as a reflection of oxygen lack) they all miscarried. Therefore I would suggest that a second good source of material would be the abortuses of such patients with cyanosis. In the meantime I think it would be very dangerous to extrapolate from rodents at 30 000 feet to humans who reproduce at much lower altitudes than that.

T. INGALLS : The only comment I would make is that at Morococha and at Cerro de Pasco, and in places like these at high altitudes, I think you get high intrauterine mortalities, as you suggest. I am going to suggest also, along with Dr. Degenhardt, that there is much to be gained from retrospective studies even though they have limitations. I don't think it was on a coldy prospective basis, Dr. McBride, that you made your observations in 1960. It is sometimes amazing to see what some of these mothers can remember when things have gone wrong, and without being neurotic about their memories. I would like to ask Dr. Degenhardt if he wouldn't defend the retrospective method a little.

K. H. DEGENHARDT : I have several questions. I should like to comment first on Dr. Miller's remark concerning the value of epidemiological studies to elucidate some facts of the etiology of congenital malformations. Dr. Miller, be sure that our group has a lot of self-criticism using the method of prospective investigation. I personally must say that I think we should use all three main research methods : This means prospective, retrospective and experimental investigations; only then we may have success. We focus, too, on the time before conception and around conception. As a geneticist I know that causes of congenital malformations may be before conception but I may focus your attention on the very important load of multiple factorial caused congenital malformations. This means those malformations are caused by several genes and several external agents. I may ask you, Dr. Miller, how will you succeed with your excellent epidemiological studies which may be a very good warning system, to enter this field of very complex etiological factors of congenital malformations. I appreciate, very much, the work of Professor Nishimura. He is very close to this times, where these intrinsic and extrinsic factors act. I mean the factors which make true the derangement from the site of the genetic factors and from the site of the external agents. I introduced the problem of congenital malformations using hypoxia in pregnant rabbits and it is just a wonderful occasion to thank Professor Ingalls for his splendid ideas. He didn't know that he stimulated me to take on this work but I was clear by myself that hypoxia may not be a real teratogenic agent of importance for the human being. My intention was to look for sensitive stages in early pregnancy and I surprisingly found that the vertebral column primordia reponded in a very brief time of early gestation; I substituted the findings of Professor Ingalls that there is a cranio-caudal gradient concerning the need for oxygen in the axial primordia. I should not touch this any more.

I may focus on Dr. Yamamoto's work and I congratulate him. I am a clinician, a pediatrician too, and I know what it means to look for causes of mosaicism in chromosomal aberations. We know nothing in human beings up to now, and you may be one of the first who has arranged chromosomal mosaicism :

you found here. I may ask you what is the physical relevance in the embryos of triploidy mosaicism and of tetraploidy mosaicism. I am well aware of the problem of triploidy in rabbits. I focus on the work of Beatty and Fisherg in England. I think Professor Warkany is well aware of their beautiful study on triploidy caused by colchicine; they never succeeded to raise triploid embryos beyond the neurulation stage. But you found triploid embryos at the stage of 9 days, I know what this stage means, in the golden hamster. You have paddle formed limbanlagen on a well-formed embryo. What is physical relevance...

T. INGALLS : We think there is a survical factor here, a very large one, and that is why in all of this work he has made two samplings. The first is made during the preimplantation stage, perhaps at 3 days when the embryo has about 16 cells, something of this order; the second sampling is made 9 days after copulation. Ideally Dr. Yamamoto should have taken multiple samples of each fetus. However, technically this was too difficult and time-consuming, but the results would be very interesting. Thus, we had a specimen recently that at first seemed to be trisomy mosaic; the trisomic chromosome involved only one group. After a while, however, the cells proved to be consistently haploid. We really wish it had been possible to check back on source material to see if other sampling would have given trisomic counts at first, which later became consistently haploid.

P. MARKS : Have you followed the fetal development to term to obtain data as to percentage of anoxia induced aneuploidy and polyploidy basic detectable abnormalities ? If such data are available, what is the spectrum of abnormalities observed ?

M. YAMAMOTO : These fetuses with polyploids are determined at 9 days.

P. MARKS : How long is term, in a hamster ? 16 days ? So they are over half-way through.

T. INGALLS : You can almost diagnose the runts as triploids. I don't know whether you can diagnose others or not, by inspection.

P. MARKS : You think they die ?

T. INGALLS : Yes, a lot of them die.

M. YAMAMOTO : I don't think triploid embryos can survive throughout pregnancy. When I find triploid fetuses, they are treated like implantation sites. In the case of tetraploid mosaic fetuses they are more mature and closer to normal.

T. INGALLS : May be it will be interesting to look for a connection between what Dr. Yamamoto has just told you and what is scheduled on tomorrow's program when we will have a consideration of the very high incidence of chromosomal anomalies in abortuses. The correspondence begins to make sense.

M. WINICK : Is this more wastage or more congenital malformations ?

M. YAMAMOTO : We have found a high incidence of pregnancy wastage in test animals as compared with the control.

T. INGALLS : I think that the chromosomal anomalies and maybe even the congenital anomalies are manifestations of increased pregnancy wastage, the extreme manifestation of which, of course, is death.

R. G. WHITEHEAD : I hope you don't mind my asking a question about something I know nothing at all about, but with such a diverse audience, maybe it is permissible for people who do not know much about a subject to ask a question. It is in relation to the human significance of the animal experiments that you are doing. Other speakers have made comments on altitude, and Dr. McBride has mentioned the possible importance of anaemia, and I wondered if in fact you have measured the oxygen tension in the blood of your experimental animals and compared that with the oxygen tension in the blood of *human beings* who are anaemic ?

T. INGALLS : Well, I'll give you a reply. I can't answer the whole question because one doesn't have all the physiological parameters that he should have, and I'd have to ask Dr. Bennett for his tolerance and suggestions. The reason why these rough parameters of altitude are given is simply because they work, and there are so many variables : hamster ovulation, hamster copulation, hamster oestrous cycle that you try to keep the conditions of the experiment as simple as possible.

A. E. HELLEGERS : Might I suggest that there might be a difference between whether it is dependent on oxygen *tension* or oxygen *content*. In the problem with anaemia the oxygen tension may be quite normal, but the oxygen content quite low. The question may be whether some fetal enzymes might be activated by tension and others by content. The second thing which may be relevant in this context is that in abortuses of women with cyanotic heart diseases, the abortions can occur as late as the 12th or 13th week of gestation. A third factor which might be of importance is that these cyanotic pregnant women often have very low urinary pregnanediol excretions. I believe there is beginning to be some

evidence that the progesterone production of the placenta may be affected by the oxygen environment, so that this might be the cause of the pregnancy ending.

T. INGALLS I feel very comfortable to have my friend Stanley Bennett in the front row, because he is a physiologist and a reproductive physiologist, and maybe he can help answer these.

S. BENNETT : Professor Ingalls flatters me. I am not courageous enough to comment on the questions he raised. In retreat, may I ask that we return to the question of triploid or tetraploid embryos. I wish to remind this conference of the work of Dr. Fonkhauser, performed fifteen or twenty years ago. Perhaps Professors Ebert or Monroy or Nishimura will recall these more accurately than I and correct me if I remember them faultily. As I recall Fonkhauser's work, he succeeded in raising triploid and tetraploid salamanders. These animals were very delicate, and far less robust than the normal diploid specimens. Yet in the polyploid salamanders, the organs appeared nearly normal grossly, yet on microscopic examination, the cells were larger than in diploid ones and, by inference, there were hence cells in organs of normal size. Fonkhauser pointed out that a brain, for example, with larger and fewer cells than a normal, would probably be simpler in its connections and that hence triploid or tetraploid animals are probably more stupid than the diploid and can be regarded as mentally retarded or underdeveloped. They may well lack important control mechanisms and hence have greatly reduced viability in spite of grossly normal organ morphology. Dr. Fonkhauser's work suggests the inadequacy of looking only for gross developmental anomalies in triploid and tetraploid embryos and commends the use of more penetrating methods for searching for defects.

L. WOLPERT : It seemed to me that many of the foetal abnormalities that have been described, irrespective of their nature, lead to abortion of the foetus. This is rather surprising and I want to know if it is true, and if it is true, what is the reason.

H. NISHIMURA : I would like to point out that unlike postnatal death, detailed preceding processes of the intrauterine death are unknown. For example, although most of the polydactylous embryos are supposed to be lost during the later course of pregnancy, the cause is not clear. It may be due to that many of the externally malformed embryos are accompanied by some serious internal malformations.

J. EBERT : I have found the emphasis, throughout the day, on environmental factors, to the exclusion of the genome, disquieting. Until Professor Degenhardt's

observations, I found myself reliving the ancient history of developmental biology, a field that failed to advance until it was re-united with genetics. I would re-emphasise the need to utilise, as far as possible, inbred strains of animals providing a known genetic background for studies of the action of environmental teratogens. I would also stress the need to design experiments to answer *specific* questions. Finally, the availability of cloning and somatic hybridization techniques for human cells should make it possible to answer questions about the interaction of teratogens with the genome.

T. INGALLS : I do not think this work poses any threat to genetics as such. However, these experiments do demonstrate that events other than those which are genetically programmed can determine anomalous chromosome formations. I myself do not regard this finding as oppressive to genetics. I would like to ask Dr. Warkany if he looks at this matter the same way.

Whereas I have said I see no conflict here, Dr. Ebert asserts that our emphasis on the environment is very unfortunate. I simply beg to disagree. Ten years ago it was Dr. McBride who was taken to task for putting too much emphasis on the environment. His paper did not even get a hearing. Perhaps there is a disagreement of interpretation rather than facts. If so, this is a healthy, controversy and will be settled by future workers.

As I interpret our own work and the other studies that we have built upon, the facts begin to set apart a body of chromosomal genetics distinct from gene genetics. New approaches are needed just as they were needed when viruses were set apart from bacteria for special study. Why not ? This is not a question of environmental emphasis "to the exclusion of genetics, or genetics to the exclusion of environment". Quite the reverse; there is evidently an interplay going on between both worlds. Moreover, there seems to be a demonstrable vulnerability of particular chromosomes to change, especially during the preimplantation period about which we are just beginning to learn many basic facts. I have no hesitation in saying that critical experimentation is needed to settle point at issue and differences of opinion among investigators. This is science, the same, often inexact, science of Buffon, Lamarck, Isaac and Geoffroy St. Hilaire, Lemery and Dareste, as well as the more exact science of Mendel and of his followers in the twentieth century with our more advanced technological aids. So dependent are we on our aids that we tend to place more reliance on them than upon evidence that is directly accessible to the senses. Yet this was the evidence Mendel used; this was the evidence of Gregg and McBride. If our illustrious forebears had fewer tools to work with, they made up for it with the ebullience of their speculations and the frank acceptance of controversy and debate as a way of life. Perhaps we shrink too much from simple differences of opinion.

Dr. Ebert is critical of our failure to use inbred strains. Whereas this may be a further refinement for future investigators, I don't see that the use of inbred strains would have affected our results very much or our thinking. We were examining a species, not a strain characteristic. Indeed, I suspect we are examining a principle which goes far beyond Cricetus auratus and probably applies widely to the animal kingdom. Certainly, my coworkers and I have observed chromosomal anomalies in mice after experimentally caused (alloxan) diabetes and sheep poisoned by plant alkaloids shortly after fertilization. I can not understand why Dr. Ebert is critical of us for showing that the hamster makes a better model for such experiments than rats, mice, rabbits and sheep. Watanabe, Horii, Nogami, Shidou, Endo, Yamamoto and I have spent ten years in this work and we have no reticence about extolling the virtues of the golden hamster. It seems to me that we have learned much that is worthwhile and are going to learn more.

In the first place, this kind of experimentation puts our hypotheses on a solid scientific foundation. They are more than speculations. They can be repeated or confirmed by those who are willing to test the principle that there is an epidemiology of acquired chromosomal mutations and that germinal mutations may have their genesis from natural forces operating during the preimplantation stages of life — between ovulation, fertilization and cleavage stages. These findings also have important clinical application for numerous puz ling chromosomal abnormalities of a kind well known to pediatricians and to obstetricians, the polyploidies and aneuploidies described by Professor and Mme. Boue on December 4th. When the conceptus survives, suche chromosomal anomalies are usually accompanied by anatomical stigmas and chromosomal defects, notably Trisomy D, Trisomy E, Trisomy G, selected monosomies and aneuploidies of the sex chromosomes — XO Turner's Syndrome, the XXY complex and others.

As I see the significance of these anomalies in the aborted and miscarried conceptus, they can no longer be unquestionably accepted as the hallmark of an inferior genotype. Some may be, but most, I think, are not. My own opinion is that most of these anomalies are mutations and are usually acquired along the tortuous way between ovulation, reduction division of the chromosomes, descent of the oocyte along the oviduct, secondary meiosis, fertilization and implantation. Today we are sure that there are many opportunities for things to go wrong and we should begin to place our theories on the anvil of experimentation. This is why I have placed emphasis on the animal model that I myself wish to use for my experimentation and on the particular teratogenic and mutagenic agents to be employed. To sum up in the same words I used in my prefatory remarks to Dr. Yamamoto's paper "We feel sure that a whole system of chromosomal

anomalies has been uncovered that have their origins at one or more of the stages critical for normal reproduction".

Another area where there will surely be an important impact of this kind of experimentation is ecology and natural history, in particular a better understanding of chromosomal mutations as being a direct response to naturally occuring agents, especially heat, cold, hypoxia and critical shifts in the acid base balance. Progress here assuredly affects our understanding of evolution as having not only its gene components but also having great contributions from chromosomal changes. These appear likely to me at least to underlie the sudden evolutionary leaps or " sauts " as our French hosts will call them, which may separate one species from another, there being no signs of transitional forms or that there had ever been such forms.

J. WARKANY : I think your experiments and Dr. Yamamoto's experiments, are tryng to show how chromosomal anomalies can come about by well-defined environmental causes, and that's what we need. We want to know to why older women have a greater tendency to trisomy 21. We don't know what the mechanism is and whether it is hypoxia. I believe some endocrine causes will be found that do cause older women to have anomalous ova and I think your work is a model that may have no correspondents in real human situations, but that's all right. We need such models, and I just want to end with one question. How do you prove your chromosomal anomalies in that 9-day old hamster ? What tissues do you take for analysis ?

M. YAMAMOTO : This is what I want to know, as I said before, I cut the embryos into 10 or 15 pieces, and I pick up the small pieces at random and I don't know where it came from. But now I can take a specific tissue. We use lactic acid. No colchicine treatment. A modified method of air drying.

J. WARKANY : You separate the cells by ?

M. YAMAMOTO : I disassociate the cells by means of lactic acid.

J. WARKANY : And then you stain them ?

M. YAMAMOTO : Yes. By Giemsa stain.

J. WARKANY : Giemsa stain ? And what is the chromosone count in these cells ?

M. YAMAMOTO : In the case of polyploid formation, I could count more than 250 metaphase set from one fetus.

J. WARKANY : So you actually have tissues in which you can see the chromosomal anomalies in 9-day old hamsters. That's a great achievement. We tried for a long time for methods of chromosomal anomalies in tissues of embryos and it was very difficult. So I am very happy to hear that you can do it, and I would like to learn the method.

M. YAMAMOTO : We tried many methods before we discovered such method, trypsinisation, mechanical dissociation. Finally I used lactic acid.

Journée du 3 décembre 1971

Première séance

STRATÉGIE SCIENTIFIQUE

PRÉSIDENT RAYMOND TURPIN

RÉFLEXIONS SUR LA PROPHYLAXIE DES EMBRYOPATHIES

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Les anomalies congénitales figurent parmi les épreuves les plus redoutables. Leurs conséquences sont morales et matérielles.

Si, en effet, elles sont source de transcendance, elles risquent fort de troubler l'épanouissement familial, car l'attention de la famille se concentre sur l'enfant déshérité. Les soins qu'il faut lui prodiguer accroissent les charges matérielles.

Souvent, avec un dévouement admirable, les parents des enfants inadaptés participent bénévolement à la création et au contrôle des centres médico-pédagogiques et des ateliers dirigés, indispensables à leur traitement.

Loin de se désintéresser de ce problème démographique, l'Etat, les municipalités accroîssent progressivement leur appui matériel. Certaines industries s'intéressent aussi aux possibilités des ateliers dirigés.

Notre éance a pour objet les *anomalies congénitales,* géniques ou chromosomiques.

Leur *étiologie* peut être classée chronologiquement :

1°) En premier lieu figurent les facteurs antérieurs à la procréation. Ce sont les *facteurs progenésiques* [1]. Les plus importants sont mutagènes.

A ce propos, il convient de rappeler qu'il existe un critère révélateur de *mutations géniques* létales liées à l'X. Ce critère n'est autre que les variations du rapport du nombre des nouveau-nés masculins à la totalité des naissances, c'est-à-dire la masculinité ou sex-ration [2].

Chez la femme irradiée le gène létal lié à l'X, s'il est récessif, ne réduit que le nombre des nouveau-nés masculins. Il diminue donc la sex-ratio. Dominant, il agirait de même sur les deux sexes sans entraîner de différences. Chez l'homme irradié, le gène létal dominant peut seul réduire le nombre des nouveau-nés féminins. Il accroît la sex-ratio. Cet effet est indépendant du délai irradiation-conception, ce qui laisse entendre que les spermatogonies sont en cause.

Ces conséquences de la radiothérapie pelvienne, significatives chez les femmes, concordent avec celles qui furent observées parmi les populations exposées aux bombardements atomiques de Nagasaki et Hiroshima. Il est très probable que certains avortements spontanés sans aberrations chromosomiques sont la conséquence de gènes létaux méconnus.

D'autres facteurs progenésiques sont ceux qui préparent les *mutations chromosomiques.*

Les uns, rares, sont déjà inscrits dans le patrimoine héréditaire de l'un des progéniteurs, aberrations de structure compensées et latentes, ou bien aberrations limitées aux gonades, qui paraissent jusqu'à présent exceptionnelles. Mais le fait le plus courant est l'aberration numérique ou structurale qui survient lors de la gamétogenèse masculine ou féminine.

2°) En second lieu, ce sont les facteurs contemporains de la fécondation qu'il nous faut étudier; ce sont des facteurs qu'on peut appeler *génétiques.*

Quelques observations cytogénétiques laissent entendre que des anomalies du délai physiologiques ovulation-fécondation peuvent entraîner dans l'espèce humaine des accidents zygotiques ou embryonnaires observés de longue date dans les espèces animales. Le vieillissement de l'ovule ou des spermatozoïdes dans les voies génitales de la femme pourrait provoquer, d'après quelques exemples, des trisomies ou des triploïdies.

Toute irrégularité des cycles menstruels après grossesse, avortement ou usage d'anovulatoires comporterait des risques embryonnaires qu'il serait bien utile de préciser.

3°) En troisième lieu, les *facteurs métagenésiques* sont les *agressions* virales, chimiques, physiques qui atteignent l'embryon. A cette phase encore peuvent survenir des aberrations chromosomiques. Très précoce, blastomérique, un trouble de ségrégation peut réaliser un blastomère triplo 21, par exemple, qui donnera un caryotype homogène, l'autre blastomère haplo 21 étant inviable à l'état homogène. Plus tardif ce trouble donnera un mosaïcisme.

4°) Parmi ces facteurs étiologiques, une place doit aussi être réservée à la *prédisposition héréditaire* possible aux aberrations chromosomiques. La génétique fondamentale nous en donne des exemples, en particulier chez la drosophile.

La pathologie humaine attire parfois l'attention sur la coïncidence familiale d'aberrations chromosomiques ou d'aberrations chromosomiques et de gémellité, ou d'états pathologiques tels que les leucoses, les cardiopathies congénitales.

Certains stigmates héréditaires dominants, en particulier dermatoglyphiques,

sont plus fréquents de façon significative chez les parents de trisomiques 21. L'on est en droit de se demander si une telle prédisposition n'est pas en cause lorsqu'apparaît dans une famille jusqu'alors indemne d'aberrations chromosomiques un trisomique 21 sans qu'on puisse invoquer l'âge maternel ou une translocation latente chez un des parents.

Un second problème fondamental est la *séméiologie embryonnaire précoce*, celle qui pourrait identifier sans réserve les embryopathies des trois premiers mois.

1) Les *conjonctures familiales* qui permettent d'affirmer l'embryopathie sont exceptionnelles [3].

Embryopathie chromosomique quand une translocation latente entre chromosomes homologues du père ou de la mère, le chromosome 21 d'après des faits connus, ne peut donner que deux types d'embryons pathologiques, l'un triplo 21, mongolien viable, l'autre haplo 21, homogène, éliminé par avortement spontané.

Embryopathie génique quand les procréateurs, sourds muets ou aveugles, par exemple, sont l'un et l'autre des homozygotes récessifs, autosomiques et concordants.

2) Quant aux *signes de présomption*, ils sont nombreux mais ils n'ont qu'une valeur très relative [4].

Age maternel, fréquences des avortements spontanés des premières semaines, métrorrhagies. L'étude précoce et répétée des métabolites urinaires a fait naître l'espoir d'informations sur la vitalité du fœtus et la qualité de la fonction placentaire. Il n'est donc pas étonnant que dans les pays où l'avortement sélectif des embryopathies graves et incurables est légal, les possibilités de dépistage d'aberrations chromosomiques, d'anomalies biochimiques par l'amniocentèse aient été utilisées. Il est à souhaiter que cette technique gagne en innocuité et en précision.

3) Le très grand intérêt scientifique de cette séméiologie embryonnaire, est de préciser les conséquences d'aberrations de nombre ou de structure rapportées à des chromosomes particuliers.

En effet, s'il existe des analogies entre ces souffrances d'embryons qui commencent seulement à se différencier au début du deuxième mois, mois de l'organogenèse, la séméiologie anatomique de l'embryon avorté car inviable, ou anatomo-clinique et biochimique de l'embryon viable, traduit une *certaine spécificité chromosomique*.

Elle met aussi en valeur des stigmates jugés auparavant comme négligeables,

tels les troubles dermatoglyphiques dont la valeur diagnostique pour la trisomie 21 est incontestable.

4) Mais il ne faut pas croire que le but final du dépistage des embryopathies des trois premiers mois n'est autre que de provoquer un avortement sélectif là où la loi l'autorise.

Développer ses possibilités aurait surtout l'énorme intérêt de rassurer les mères qui, terrifiées par la perspective d'un enfant anormal, justifient en raison de désordres mentaux qui mettent leurs jours en danger, un avortement thérapeutique. Celui-ci est autorisé en France par la loi, mais il risque bien souvent de tuer un embryon normal. A l'heure actuelle 50 pour cent des avortements thérapeutiques dans la région parisienne seraient motivés par le danger mental et non corporel qui peut menacer la vie de certaines femmes enceintes.

Une dernière remarque s'impose à notre attention. En approfondissant les causes des avortements spontanés, en révélant que 50 pour 100 d'entre eux durant les dix premières semaines de l'embryogenèse sont déterminés par des aberrations chromosomiques, la cytogénétique, l'anatomie pathologique, la virologie qui sont au programme de cette séance ont précisé la *valeur de cette sélection naturelle* [5]. Les avortements spontanés ne protègent-ils pas, en effet, l'espèce humaine contre des embryopathies incurables. Et notre devoir n'est-il pas de respecter cette protection naturelle ?

Une enquête sur la fréquence des tares congénitales manifestes observées dans trois maternités parisiennes de 1941 à 1950 révéla sur 79 844 naissances, 674 nouveaux-nés malformés [6]. La séparation de ce lot en deux groupes, celui des enfants nés en période de graves troubles socio-économiques de 1941 à 1946, et celui des enfants nés de 1947 à 1950 en période de retour à des conditions de vie normale, mit en valeur une différence significative entre ces deux groupes.

La quantité des anormaux graves pour 1 000 de 1941 à 1946 s'élevait à 7,38; de 1947 à 1950 à 9,27.

Cette différence de 7,38 pour 1 000 à 9,27 pour 1 000 prend toute sa valeur si l'on remarque en outre que la dissemblance pendant ces périodes de l'âge moyen des mères et du rang de naissance moyen de leurs enfants aurait dû contribuer à un résultat inverse :

âge moyen pour la période 1941-1946 : 26,65 ans;

âge moyen pour la période 1947-1950 : 25,90 ans;

rangs moyens de naissance respectivement de : 2 198 et 2 064.

Nous avons donc estimé que cet accroissement considérable de 25 pour cent pouvait traduire l'influence de l'amélioration des conditions de vie et des soins médicaux sur la tolérance des mères vis-à-vis d'œufs pathologiques.

Un processus analogue pourrait expliquer que la fréquence des aberrations gonosomiques décelables par la recherche du corpuscule chromatinien, notée parmi les nouveau-nés européens, n'ait pas été retrouvée parmi les nouveau-nés d'un pays économiquement moins favorisé. Cette hypothèse justifierait une étude parallèle des avortements spontanés.

En conclusion, le fléau congénital par sa gravité et son expansion est aujourd'hui le problème le plus important de la médecine préventive.

En le mettant à l'ordre du jour de cette réunion de la Fondation 41 de l'Institut de la Vie, le Professeur Maurice Marois et le Dr McBride ont pris une initiative dont nous devons leur savoir gré.

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ETUDE DES ABERRATIONS CHROMOSOMIQUES DES AVORTEMENTS SPONTANEES

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Je vais passer en revue les différents thèmes qui nous ont retenue dans nos recherches et il sera possible au moment de la discussion si certains thèmes intéressent plus les uns ou les autres de revenir dessus et de les discuter plus à fond.

Les recherches de notre Laboratoire de Longchamp sont orientées vers la détection et la prévention des malformations congénitales.

Depuis la découverte que certaines malformations des enfants, telles que le mongolisme, sont liées à la présence d'un chromosome en surnombre, il devenait raisonnable de penser qu'un certain nombre d'avortements spontanés pouvaient également avoir pour origine une anomalie chromosomique, d'autant plus que les études morphologiques et anatomopatholigiques faites en particulier aux U.S.A. et au Canada avaient montré que la majorité des avortements précoces étaient dus à une malformation du zygote et que sur la totalité des grossesses terminées avant terme, 25 pour cent correspondaient à un fœtus pathologique.

A la naissance, les malformations liées à une anomalie chromosomique sont rares, ne représentent que 1 % environ des naissances et ne peuvent pas être analysées dans une étude unique. Par contre, l'étude des avortements spontanés permet de rassembler dans une seule et même enquête un grand nombre d'observations conduisant à des analyses statistiques et épidémiologiques.

Nous nous sommes attachés à étudier les avortements du premier trimestre de la grossesse. La limite de trois mois n'est pas arbitraire mais elle est imposée par les faits; car 80 % des avortements spontanés se situent à cette période et le études morphologiques ont montré que 80 à 90 % des œufs expulsés à ce stade sont malformés. Enfin, au-delà de cette limite le corps jaune maternel ayant terminé son rôle de maintien de la grossesse, si l'œuf n'a pas acquis une autonomie hormonale, il est éliminé.

L'examen chromosomique de plus d'un millier de specimens nous a donné une fréquence de 60 % de caryotypes anormaux. Cette fréquence doit être interprétée comme une fréquence minimum car il a certainement été inclus involontairement dans cette étude au début en particulier un certain nombre d'avortements provoqués. L'examen anatomique des œufs abortifs a révélé que la fréquence des anomalies chromosomiques varie avec le moment où l'œuf s'est arrêté dans son évolution. Plus l'arrêt est précoce, plus le nombre d'anomalies est élevé allant de 70 % à trois semaines, à 65 % vers six semaines, et seulement à 20 % dans les spécimens dont le développement est de sept à douze semaines.

TABLEAU 1

Fréquence des anomalies chromosomiques en fonction de la durée du développement du zygote$_2$ (965 *observations*).

	durée du développement en semaines										
	2	3	4	5	6	7	8	9	10	11	12
nombre total caryotypés	17	310	186	106	227	37	27	33	9	7	6
nombre avec anomalies	9	208	105	64	156	17	4	5	6	0	2
fréquence	65 %	67 %	62 %	64 %	69 %	46 %	15 %	16 %			
			558/846				17/82				
			66 %				20 %				

La presque totalité des aberrations chromosomiques sont des anomalies du nombre des chromosomes correspondant donc à un accident de répartition des chromosomes parentaux au moment de la fécondation : les parents ont des formules chromosomiques normales. Dans 3 % des cas seulement, nous avons trouvé des anomalies de la structure chromosomique transmises dans la moitié des observations par un des parents.

TABLEAU 2

Fréquence des diverses anomalies chromosomiques (665 *observations*).

anomalies du nombre des chromosomes	*Nombre*	*fréquence*
monosomies X	101	15,2 %
G	1	
trisomies	355	53,4 %
doubles trisomies	11	1,6 %
triploïdies	137	20,7 %
tetraploïdies	37	5,5 %
anomalies de structure	23	3,3 %

La mesure de l'importance de la fréquence des anomalies chromosomiques de ces avortements doit conduire le gynécologue à peser l'intérêt de certains traitements hormonaux en cours de grossesse, et à ne pas intervenir dans certaines menaces d'avortement qui sont le plus souvent le rejet naturel d'un embryon malformé.

Dans 30 % des observations, l'anomalie trouvée dans l'avortement est une anomalie identique aux anomalies décrites à la naissance chez des enfants malformés. A travers l'étude des avortements, il devrait être possible de comprendre pourquoi certaines aberrations conduisent à un avortement précoce et pourquoi apparemment la même anomalie peut aller à terme.

Dans des cas particuliers, nous avons remarqué que la mort embryonnaire n'était pas due à des malformations de l'embryon plus importantes que celles observées à terme, mais que le placenta s'arrêtait de croître et donc d'assurer sa fonction précocement, conduisant secondairement à la mort de l'embryon. Les cultures cellulaires faites à partir de ces avortements nous ont montré parallèlement que les souches cellulaires avec anomalies avaient des divisions cellulaires très ralenties par rapport aux souches avec caryotype normal. Le même ralentissement de la croissance du placenta est observé au tout début de la grossesse, mesuré par des dosages hormonaux répétés, dans les grossesses en évolution et qui aboutissent à la naissance d'enfants malformés.

Cette surveillance hormonale semble un moyen de détection précoce des anomalies et devrait permettre de sélectionner les femmes à haut risque chez lesquelles une ponction de liquide amniotique faite vers trois mois de grossesse, se justifie et permet de s'assurer du caryotype du fœtus.

Dans notre étude des avortements, nous avons cherché à déceler des facteurs pouvant favoriser la formation de ces anomalies chromosomiques. L'avortement se situant très tôt après la fécondation, on peut espérer collecter des informations sur les circonstances de la fécondation. Nous avons en particulier étudié la qualité des cycles menstruels : certaines anomalies surviennent plus fréquemment chez les femmes ayant des cycles longs avec ovulation tardive, en particulier les triploïdies. Des délais entre l'ovulation et le rapport fécondant sont significativement déterminants, soit vieillissement du spermatozoïde dans les voies génitales de la femme avant l'ovulation, soit vieillissement intrafolliculaire de l'ovule ou ovulation normale mais fécondation tardive sur un ovule émis depuis un temps trop long.

Dans le tableau 3, nous avons rassemblé 139 observations dans lesquelles nous pouvions connaître la date de l'ovulation de la femme par une courbe de température et connaître les rapports fécondants. Dans une certain nombre d'observations, il s'agit d'un rapport unique et quand il y a eu plusieurs rapports, nous avons jugé comme rapport fécondant celui qui était le plus près

TABLEAU 3

Délai ovulation-rapport fécondant (139 *observations*).

délai en jours	nombre d'observations	nombre d'anomalies		fréquence
6	1	1		
− 5	3	3		
− 4	3	2	45/50	90 %
− 3	6	6		
− 2	16	14		
− 1	21	19		
0	65	43		66 %
+ 1	8	7		
+ 2	8	6		
+ 3	4	3	19/24	79 %
+ 4	2	2		
+ 5	1	1		
+ 6	1	0		

de la date de l'ovulation. Nous avons été assez surpris de trouver des chiffres avec des différences significatives parce que même un vieillissement de 24 heures, que ce soit avant ou après l'ovulation paraît être déterminant pour les anomalies chromosomiques, en particulier quand le spermatozoïde a vieilli dans les voies génitales avant la fécondation, le chiffre des anomalies de 90 % est significatif. Le vieillissement de l'ovule paraît un tout petit peu moins déterminant. L'influence de l'âge maternel ne paraît significative que pour quelques anomalies, qui sont les mêmes que celles qui vont à terme : mongolisme et trisomie D., les autres anomalies survenant chez des femmes qui ont le même âge que des femmes menant à terme des enfants normaux.

TABLEAU 4

cause de l'arrêt de l'ovulation	délai avant la conception	avortements avec anomalies						total
		ensemble		trisomies		polyploïdies		
grossesse et accouchement	0- 6 mois	16	64 %	11	44 %	3	12 %	25
	7-12 mois	22	69 %	12	38 %	6	19 %	32
grossesse et avortement	0- 6 mois	47	68 %	29	42 %	11	17 %	69
	7-12 mois	43	62 %	25	36 %	15	22 %	69
contraceptif oral	0- 6 mois	43	68 %	23	36 %	13	20 %	63
	7-12 mois	18	62 %	11	38 %	3	10 %	29
groupe témoin		256	62 %	134	32 %	78	19 %	413

Vous voyez en bas du tableau le chiffre en France des femmes qui mettent au monde des enfants normaux et à terme; il est de 27 ans et demi; c'est le chiffre que nous retrouvons pour les monosomies X. Pour les triploïdies et les tétraploïdies, elles sont au contraire plus jeunes. Par contre, les trisomies dans l'ensemble ont un âge moyen plus élevé mais la différence n'est réellement nette que pour les trisomies D et les trisomies G. Les autres trisomies ont une très légère augmentation de l'âge maternel, mais qui est beaucoup moins nette.

L'influence des irradiations du père ou de la mère, ou des deux est déterminante. Par contre, nous n'avons pas remarqué une influence des arrêts de l'ovulation par contraceptifs oraux, par comparaison avec des arrêts physiologiques de l'ovulation par grossesse — ou avortement se situant dans l'année qui précède la fécondation de l'œuf abortif.

Nous avons trouvé une influence significative du tabac, mais inverse, dans ce sens que les grandes fumeuses qui ne s'arrêtent pas de fumer après la fécondation ont une augmentation significative des avortements avec caryotype normal. Donc il est vraisemblablement intervenu un facteur externe conduisant à une augmentation des avortements avec caryotype normal.

Cette revue rapide des facteurs favorisant les anomalies chromosomiques que nous avons reconnus, permet de voir qu'il paraît difficile d'instituer une prophylaxie à ce niveau. Par contre, il devrait être plus facile soit de faciliter le rejet qui fonctionne déjà à 99 % dans le cas des anomalies chromosomiques conçues, soit de surveiller plus étroitement les grossesses par des dosages hormonaux répétés afin de définir le risque.

La survenue d'un avortement étant beaucoup moins traumatisante pour la femme que la naissance d'un enfant malformé, les motivations vis-à-vis des grossesses ultérieures ne sont pas les mêmes. Il nous a été possible de suivre les événements obstétricaux dans ce groupe de femmes dont nous avions examiné un avortement avec caryotype normal ou non : 500 femmes ont été suivies. Nous avons d'abord jugé leur fécondité : 80 % des femmes de 20 à 25 ans ont eu une nouvelle grossesse quand le recul est de deux ans après l'avortement examiné et 35 % des femmes de plus de 40 ans.

La fréquence des avortements ultérieurs est de 25 % dans le groupe où le caryotype de la première fausse-couche avec caryotype anormal chez une femme jeune doit conduire à porter un bon pronostic pour les grossesses suivantes. Nous n'avons trouvé que 10 % d'avortements, ce qui est moins que des témoins (15 %).

Les enfants nés après un avortement avec anomalie chromosomique étaient tous normaux. Nous avons pu caryotyper une trentaine de deuxièmes fausses-couches, et nous avons remarqué — et la différence est hautement significative — que lorsque la première fausse-couche a un caryotype normal, la deuxième

a également un caryotype normal. Par contre, quand la première fausse-couche a une anomalie chromosomique, la deuxième a également une anomalie chromosomique : il s'agit, d'ailleurs le plus souvent d'une anomalie différente de la première.

Il s'en suit d'un point de vue pratique que si une femme a une anomalie chromosomique dans un avortement, elle a beaucoup de chances d'avoir un enfant ensuite et que si elle fait une menace d'avortement, il ne faut certainement pas la traiter car elle a plus de risques que ce soit de nouveau une anomalie chromosomique.

En conclusion, d'un point de vue fondamental, l'étude des avortements est une étape dans la connaissance des erreurs cellulaires au moment de la fécondation. Les analyses mathématiques des avortements permettent de penser — et là nous rejoignons les études démographiques et gynécologiques — qu'environ une fécondation sur deux aboutit à un zygote non viable, certaines anomalies étant rejetées avant l'implantation de l'œuf, d'autres permettant l'implantation mais pas d'embryogenèse, d'autres conduisent à des défauts de l'embryogenèse.

Les cultures cellulaires de produits d'avortements permettent de constituer des souches cellulaires porteuses de différentes anomalies chromosomiques qu'on ne voit jamais en dehors des avortements. Ces souches congelés peuvent être conservées et sont un instrument de travail dans un grand nombre de domaines : enzymologiques, génétiques, immunologiques.

D'un point de vue pratique, la meilleure connaissance de la fréquence des anomalies chromosomiques doit conduire l'obstétricien à préserver le rejet naturel des embryons malformés. L'étude clinique et biologique des grossesses doit permettre sur des critères établis à partir de l'étude des avortements de définir les grossesses présentant un risque de fœtus pathologique.

DISCUSSIONS

C. LEVINTHAL : Can you give any quantitative data on the rate at which trisomies are induced by radiation ?

P. MARKS : What is the evidence that abnormal caryotypes are causally related to abortions rather than an abnormality induced by as yet unindentified etiological factors causing the abortion ?

A. BOUÉ : In many chromosomal aberrations the development of the embryo stops very early and there is often no embryogenesis at all; these aberrations are always lethal. But in other cases, especially when chromosomal aberrations are identical to the chromosomal aberrations observed at birth, the malformation of the embryo cannot explain the abortion; delay between the growth of the placenta and the morphological development of the embryo is observed, for instance the embryo is about six weeks of development and the placenta is only of four weeks development. In the same way, in cell-lines established from the embryo with chromosomal aberrations, there is a reduction of the replications of the cell population. In these chromosomal aberrations, the causes of the abortions and the causes of the arrest of the development of the embryo seems to result from placental causes.

And the second point is why are there some women who have the first time an abortion with chromosomal aberration and the second time again an abortion with abnormal chromosome ?

J. G. BOUÉ : Je crois qu'il y a d'abord un groupe de femmes qui a des avortements de causes maternelles; à ce moment-là, le caryotype est normal; les causes maternelles sont soit une béance du col, soit une malformation utérine. Dans un deuxième groupe de femmes, il est impossible de trouver des causes maternelles par un bon examen gynécologique; chez ce groupe de femmes, ce sont des malformations zygotiques qui, chaque fois, se reproduisent; c'est une répétition des anomalies chromosomiques, mais le type d'aberrations observé est différent et semble se faire au hasard.

K. HIRSCHHORN :

1) Have you been able to confirm the observation of Carr in Canada that oral contraceptives increase the frequency of polyploidy in spontaneous abortions ?

2) In the small number of structural chromosomal aberrations, what has been your experience in subsequent pregnancies ?

J. G. Boué : Je vais répondre à la deuxième question pendant qu'on prépare les projections qui répondent à la première. Dans les anomalies de structure, nous avons trouvé chez la même femme qui portait une translocation DD, deux fois des avortements où successivement on trouvait une translocation DD ayant conduit à des trisomies D.

Pour la deuxième question relative aux contraceptifs oraux, nous avons fait la même étude que David Carr avait faite au Canada en essayant de savoir s'il y avait une incidence des contraceptifs oraux sur la formation des polypoïdies, et nous n'avons trouvé aucune différence.

Le tableau 1 est le tableau relatif à la fréquence des anomalies après contraceptifs oraux comparée à la fréquence quand il y avait eu, dans l'année qui précédait la fécondation, un arrêt physiologique de l'ovulation soit par grossesse, soit par avortement. On voit qu'il n'y a aucune différence significative entre un arrêt physiologique de l'ovulation et un arrêt par contraceptifs oraux quand le nombre d'observations est suffisant.

Dans la projection suivante, je vais répondre plus directement à la question de M. Hirschhorn, c'est-à-dire la fréquence des polyploïdes. C'est toujours la différence entre le groupe témoin et les groupes de femmes qui ont eu un arrêt de l'ovulation par grossesse, avortement ou contraceptifs oraux et selon les délais. Nous n'avons vu aucune différence significative de l'augmentation des polyploïdies sur un assez grand nombre.

A. E. Hellegers : I have two questions :

1) In your data postulating sperm ageing as the cause of anomalies, could you show us a linear relationship between percentage of anomalies and length of interval between coitus and temperature rise where the pregnancy does not end in abortion ?

2) Must one accept that the temperature rise reflects ovulation immediately, or could there be a variable time interval between ovulation and température rise ? If the latter could occur, could it then be that poor ova cause late temperature rises ? Thus an aberration ascribed to a poor sperm could be due to a poor ovum, with the long interval between coitus and "ovulation" being an artefact caused by delayed temperature rise ?

A. Boué : To the first question : we haven't observed a linear correlation between ageing of sperm and the percentage of chromosomal aberrations. It is surprising

on these tables that even when the time between the coïtus and the ovulation is one day, the percentage of chromosomal aberrations is 90 per cent.

To the second question, I have no answer.

W. McBRIDE : In work done in Sydney we have been unable to find a greater increase in chromosomal abnormalities in abortuses of women who have taken oral contraceptives than in a control series.

K. H. DEGENHARDT :

1) What are the experiences concerning chromosomal abnormalities in the case of habitual abortions without uterine anomalies of the mother ?

2) What is the physical status of the embryo in the case of chromosomal aberrations ?

J. G. BOUÉ : Pour la première question qui concerne l'avortement habituel, je crois que je vais prendre le point de vue qui est celui de Melvin Kerr, en Angleterre qui pense que cela n'existe peut-être pas, parce qu'il a fait une étude très approfondie pour essayer de retrouver ce qu'on appelle l'avortement habituel, et il a une suspicion dans 5 % seulement des cas et je crois que notre étude rejoint son étude, c'est vraiment extrêmement rare de voir l'avortement dit habituel.

On n'a pas de réponse pour la deuxième question.

T. INGALLS : Is there an optimum relation of coitus to ovulation for couples within fertility problems ?

J. G. BOUE : La seule recommandation que je fais à une femme qui demande le meilleur moyen d'avoir un enfant normal, je lui dis qu'elle ne prenne pas sa température, qu'elle ne note pas les rapports sexuels et qu'elle aille à la campagne avec son mari !

C. LEVINTHAL : I have two questions, one of which is : is your data compatible with the hypothesis that all trisomies are equally frequent and that the ones, one sees, are simply a matter of selection by virtue of abortion.

And secondly, I don't think I quite understood the discussion as to linearity with delay, since you were looking at spontaneous abortions and the number had already reached 90 %, I dont't understand what linearity would mean ? I mean the statement is not rationally useful since you are already so high and it seems to me the question of the percentage is a very important one, but in a sense it cannot be answered by this data because the numbers are so high to start with.

A. Boué : For the first question : the relative percentage of the different trisomies in Carr's studies, in our's and in all the other series are very close. But what does that mean ?

This is what is observed in the material received and which grow in tissue culture; one thing which is very interesting is that the trisomy 16 which is never seen at birth is very, very frequent in chromosomal aberrations; in our series it is as frequent as the monosomy X.

When we start a cell-line with these embryonic cells, we are absolutely unable to establish a cell-line; after medium you have, when you nurse your cells, they stop to grow. That means that with this chromosomal aberration, these cells are unable to divide many times. And you know one interesting thing is that there is a good correlation between the *in vivo* life-time of these chromosomal aberrations, *in vivo* from the fertilization and *in vitro* life-time.

A. E. Hellegers : I asked my first precisely because I am not sure that a study starting with an abortion group can lead to conclusions about sperm ageing. If more than one day old sperm were to cause abortion or anomalies I suppose one must advise 40 years olds to have intercourse every day, which is a tiring concept. It seems to me one must also have a distribution of coitus-ovulation intervals, in which the pregnancy did *not* end in abortion before we can draw conclusions about the significance of time intervals. The starting point of the study should therefore be *all* temperature charts and not just temperature charts of abortions. Moreover one must no expect among temperature takers many cases of intercourse on the day of temperature rise, since they usually take their temperatures for purposes of family planning.

A. Boué : You know, when we ask these questions for every specimen we receive, we ask the parents to fill a very big questionnaire and this was one of the many questions we asked. At the beginning, we never supposed that this would be significative; we were the first surprised to find that these results which for the statistician's point of view are significative, and we were the first surprised because it seems that this mean of a Questionnaire is very primitive; when you ask to people what was the day of ovulation, what was the day of intercourse, and these are our results now, today, and I don't suppose this is the only study on man. I don't know of another study on human beings. We cannot draw very big conclusions — on that I agree with you — but these are our results.

W. McBride : What percentage of women took basal body temperature charts in the pregnancy which resulted in abortion ?

A. BOUÉ : About one woman in four of those who had had a specimen studied had taken their temperature regularly. In France, this is very popular, only because people are catholic and they do contraception with temperature control.

L. WOLPERT : Considering the number of different chromosomal abnormalities do you ever find malignancies ?

A. BOUÉ : In this study we have established around 60 cell-lines with chromosomal aberrations. We have carried about 20 to 30 cell-lines as far as possible in time, till the time of senescence of these cell-lines and we never observed a transformation in these cells.

J. G. BOUÉ : Once we found one cell-line from one foetus, the cells of which were not growing in mono-layers but they were growing in three dimensions. This was suspected to be a malignancy but we were not able to have the history of the mother.

O. HECHTER : In the case where spontaneous abortion is associated with dissociation of growth between the placenta and the embryo, does this imply that the embryo influences in some way the placenta (or the corpus luteum) as well as the reverse ?

A. BOUÉ : In all these spontaneous abortions, the time of gestation is about the same in all cases. It is thirteen weeks after the last menstrual period, with the different types of chromosomal aberrations, this is the time when the maternal hormonal secretions stop. At the beginning the pregnancy is maintained mainly by maternal hormonal secretions and then by hormonal secretions from the zygote; in chromosomal observations the zygote stop many weeks before, and you have an abortion, because there is not any more hormonal secretion.

J. G. BOUÉ : May I put a question to Dr. Winick ? I have seen that you did not agree when I spoke about the slow growing cells in placenta. May I ask you why you did not agree ?

M. WINICK : Since placenta is a fetal tissue there is no reason to assume that if changes occur within the placenta leading to a faulty placenta growth and that if these changes are the same as those in the fetus, that the fetal growth retardation is secondary to placental insufficiency. Whatever is causing the retardation may be causing it in *all* fetal tissues and the manifestations may therefore be present in *both* fetus and placenta.

V. INGRAM : Did I understand it correctly that one out of every two fertilizations leads to a non-viable embryo, or did I mistake that ? If you said this, I wonder what is the basis for that statement ?

A. BOUÉ : The basis for that statement is first the work of Hertig in pre-implantation when he found that more than 30 per cent of the eggs which he recovered in the very first days were abnormal. The second point is, for each trisomy observed, statistically you must have a monosomy. There was a lot of work done on chromosomal aberrations in spontaneous abortions, something around 1 300 or 1 400 anomalies are known, there are two or three autosomal monosomies described and there are around 600 trisomies; then in some groups of trisomies very few specimens were observed; it is mainly because they stop so early that you are unable to recover that material. So when you collect all these data, around one conception out of two is abnormal. The same is found in horses; there is 48 % fetal loss.

T. INGALLS : The 50 % mortality judging by the experimental animal (hamster) timing of coitus is, expectedly, very important in relation to health (karyotype) of conceptus.

A. BOUÉ : These observations are in small mammals. In these small mammals, the time of the maturation of the oocyte is very short and comparisons with human beings are difficult because hormonal control of the ovulation is different. In rabbit, the ovulation is after coïtus only; in most of the animals you have oestrus; in human beings there is a different physiology of the regulation of the ovulation, so the comparison is very difficult.

C. LEVINTHAL :

1) Is your data consistant with the hypothesis that the primary induction of trisomies is equally frequent for all chromosomes ?

2) Do you know if that all the cells of a fetus have the same trisomy ?

A. BOUÉ : Yes, every time it was possible when we receive those specimens and when the specimen is complete, we systematically grow in tissue culture, tissue from the embryo itself, tissue from the amniotic membrane, and tissue from the embryonic chorio, and in each specimen in which we were able to have the three tissues growing in tissue culture and to caryotype the three tissues. We always had the same caryotype, in all the cells. We have only two observations of mosaicism.

J. EBERT : To the extent that your estimate of 50 % mortality is based on the earlier observations of Hertig and others, it may be in error, for embryos recovered a number of years ago were usually obtained following interventions of pregnancy for specific medical reasons. One would have expected a high incidence of abnormalities.

K. HIRSCHHORN : I would take the opposite view of Dr Ebert. I believe that the estimate is low. First, we miss all the monosomies which must be as frequent as the trisomies due to meiotic non-disjunction. These may be resorbed, as probably is the case in Dr Ingalls' animals. A recent statistical analysis in Dr Neel's department of the University of Michigan has estimated that 75 % of fertilized ova are lost.

S. BENNETT : The physiology of mating and fertilization in fowls, frogs and fish is so different from mammals that one cannot draw inferences from the lower forms on the subject at hand. Professor Ingalls has emphasized the critical nature of the timing of mating as related to ovulation in small mammals, where the time of ovulation can be ascertained with fair accuracy. But we cannot assign a time to ovulation in women with the same accuracy. The absence of a reliable zero point in time of ovulation makes impossible an exploration of the quantitative nature of the effect of timing of coitus on fetal chromosome abnormalities in humans. In humans, the time of mating is random, with respect to the time of ovulation. Rence many matings can be expected at times which are not optimum for normal development of the fetus. Thus a high proportion of non-viable fertilizations must be expected. The work of Madame et Monsieur Boué calls attention to the effective mechanism for elimination of abnormal embryos in the human. It seems to me that it is reasonable to accept then their hypothesis that half or more of human zygotes are not viable.

R. TURPIN : Ces très intéressantes interventions ne doivent pas nous faire oublier que nous allons entendre dans un instant A. BOUÉ, à son tour.

Mais avant de clore cette discussion, je voudrais demander à Madame BOUÉ si elle peut répondre aux deux questions suivantes : en premier lieu, peut-elle nous fournir une indication sur la fréquence des aberrations latentes qu'elle a pu déceler chez les progéniteurs de ces embryopathies chromosomiques ? En second lieu, je voudrais savoir si une meilleure discrimination, du fait d'améliorations techniques, lui a permis de trouver des embryons XXY.

J. G. BOUÉ : La première question : nous avons trouvé un certain nombre d'anomalies de structure et seulement dans un tiers des observations, nous avons

pu reconnaître que l'anomalie de structure était transmise par le père ou par la mère. Donc dans les deux tiers de nos anomalies de structure reconnues, les parents ne portaient pas l'anomalie. L'anomalie très souvent, d'ailleurs, était léthale; il y avait un grand morceau de matériel chromosomique qui avait été perdu au moment de la méiose.

La deuxième question : alors, au début de cette étude, nous ne faisions pas de différence entre tous les chromosomes C et un certain nombre de nos spécimens avaient été étiquetés comme des trisomies G. Maintenant, avec la fluorescence, les techniques dites de dénaturation, nous sommes capables dans un certain nombre de cas de changer nos diagnostics et de transformer ce qui était avant un spécimen femelle avec une trisomie G en un spécimen porteur d'un complément chromosomique XXY. Malgré tout, le raffinement des techniques ne nous fait pas beaucoup augmenter la fréquence des XXY dans nos avortements puisque nous n'en avons trouvé, en fait, que trois.

ETUDE VIRALE DES AVORTEMENTS SPONTANÉS

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Je voudrais vous parler un peu d'un sujet parallèle qui sont les connaissances sur les infections qui atteignent l'embryon et surtout les méthodes de leur étude. C'est à un médecin australien, Sir Norman Gregg, que l'on doit la notion du rôle tératogène d'un agent infectieux : le virus de la rubéole. Dans toutes les études sur les anomalies dues à l'action d'un agent infectieux sur l'embryon et dans l'application des mesures préventives, c'est la rubéole qui nous a servi de modèle. Vingt ans se sont écoulés entre les observations principales de Gregg et l'isolement du virus, huit ans entre l'isolement du virus et l'application de la vaccination. Mais si le travail sur la rubéole a été fructueux et plein d'espoir, les autres recherches sur les infections de l'embryon ont été dans l'ensemble très décevantes. Mais, je crois, il semble prématuré de conclure à la rareté de ces infections virales et l'analyse des recherches entreprises et surtout des causes de leurs échecs peut nous amener à envisager d'autres modes d'approche de l'étude de ces infections.

La première approche, celle de Gregg, a été clinique et épidémiologique : il s'agissait derattacher à une maladie cliniquement caractéristique chez la mère une anomalie aussi caractéristique à la naissance de l'enfant. Mais en fait, les infections virales qui donnent une symptomatologie clinique caractéristique sont rares : les oreillons, la varicelle, la rougeole... ce sont avant tout des infections de l'enfant et elles sont rares chez l'adulte. Aucune action tératogène de ces virus n'a pu être démontrée. En fait, la plupart des infections virales sont soit sans symptomatologie, soit avec une symptomatologie banale et commune à de nombreuses infections.

De la même façon, les anomalies à la naissance appartiennent aussi très souvent à une symptomatologie commune où le retard mental et les handicaps mentaux figurent au premier plan. Donc ceci montre les difficultés de ces études basées sur la seule observation clinique et l'épidémiologie.

Une autre voie d'approche a été l'étude réellement virologique c'est-à-dire les essais d'isolement du virus à la naissance chez les enfants malformés. Ces

études dont le modèle a encore été la rubéole ont été fructueuses, en ce sens que par exemple, elles ont permis de rattacher au virus de la rubéole certains syndromes à la naissance qu'on n'avait pas, même par la bonne clinique, rattachés à la rubéole, par exemple le purpura thrombopénique; avant la grande épidémie américaine de 1963-1964 et les examens virologiques, on ne supposait pas qu'il pouvait être dû à la rubéole.

Ces recherches virologiques ont ouvert un grand chapitre qui est celui des malformations dues au cytomégalo virus. Mais jusqu'ici toutes les autres recherches entreprises à la naissance ont été négatives.

Du fait, comme vous venez de le voir, de notre grand intérêt pour les avortements spontanés, de cet immense matériel que nous avons récolté, nous avons entrepris des études virologiques sur ces avortements spontanés pensant que des infections virales qui atteignent l'embryon seraient responsables aussi d'avortements. Cette recherche a été décevante. Evidemment, nous avons isolé le virus de la rubéole, ce qui confirmait les études épidémiologiques qui avaient été faites; nous avons isolé — et c'était un fait nouveau — le virus de l'herpès type 2. Cette observation est intéressante parce qu'elle vient confirmer les études qui sont faites actuellement sur l'herpès génital, et déjà, Nahmias à Atlanta avait remarqué que les femmes qui ont un herpès génital ont beaucoup plus d'avortements que des groupes témoins. D'autre part, on a maintenant un petit nombre d'observations de malformations à la naissance qui ont pu être rapportées à un herpès de type 2.

Mais surtout, en fait, notre travail sur les avortements spontanés et sur les recherches virologiques dans les avortements spontanés nous a permis de comprendre les limites de cette recherche. Dans tous les avortements spontanés où la cause de l'avortement n'est pas une cause maternelle, mais qui est due à une anomalie du développement du zygote — qu'elle qu'en soit la cause — le délai de rétention *in utero*, est très long, vous l'avez vu, pour les anomalies chromosomiques; c'est le même pour toutes les autres causes d'origine embryonnaire, c'est un délai qui est en moyenne de 7 semaines.

Donc, au moment de l'expulsion, au moment où on reçoit le produit d'avortement, la plupart des tissus sont morts. Il n'y a plus de cellules vivantes et vous savez qu'un virus sans cellules vivantes lui aussi est mort et que même s'il y avait un virus, il n'a pas pu survivre jusqu'au moment de l'expulsion, ce qui explique la négativité de la plupart des études sur les avortements.

Ceci est, d'ailleurs, confirmé par les études sur les mammifères domestiques. Ces dernières années, on a remarqué que des morts foetales chez les mammifères domestiques ont été aussi rapportées à des infections virales dues à des virus du groupe de l'herpès : c'est la rhino-pneumonie du cheval, la rhinothrachéite des bovidés, la maladie d'Aujezky du porc et dans tous les cas, l'état de macération

des foetus de ces animaux, au moment de l'expulsion est telle que ni les recherches virologiques, ni même un examen anatomo-pathologique n'est possible et que le diagnostic ne peut pas être fait. Et cela n'est pas parce que chez les animaux, on peut faire des études expérimentales et inoculer des virus, qu'on a pu reproduire toutes ces maladies abortives. Donc là, nous avons un groupe de virus, le groupe du virus d'herpès qui, je pense, mérite d'être beaucoup étudié chez l'homme, et en particulier l'herpès génital.

Devant les échecs de toutes ces études virologiques, une autre voie d'approche a été l'étude sérologique. On a fait des études systématiques énormes qui ont coûté des masses d'argent, en suivant des dizaines, des centaines de milliers de femmes pendant leur grossesse. Le résultat de cette étude a été pratiquement voisin de zéro.

Et, en fait, elle se heurte à beaucoup de critiques : d'abord, le choix des antigènes qu'on a utilisés pour rechercher les anticorps est, par définition, arbitraire et ne peut être que limité parce que bien qu'on ait beaucoup d'argent, il y a toujours des limites; on a donc pu manquer des types d'anticorps qui auraient été importants.

Deuxièmement, le premier prélèvement de sérum qui est fait est toujours fait la première fois que la femme vient consulter pour une grossesse, c'est-à-dire vers 2 mois et demi, 3 mois, et vous savez bien qu'à ce moment-là tout est joué : les infections les plus graves sont les infections des toutes premières semaines et donc ce premier prélèvement de sérum est insuffisant et ne peut pas permettre de déceler les infections du premier trimestre.

Troisièmement, le critère qui est retenu le plus souvent est le critère de l'ascension du taux des anticorps entre justement ce premier prélèvement et un deuxième prélèvement. Or maintenant nous savons que c'est un mauvais critère. En fait, avec les techniques sérologiques classiques, on ne peut affirmer une infection virale que lorsque dans le premier prélèvement il y a absence d'anticorps et dans le deuxième prélèvement, il y a présence d'anticorps. Lorsqu'on voit une ascension des anticorps il est impossible de dire si cette ascension est due à une infection primaire ou à une réinfection, à l'effet de rappel d'une réinfection. Et là encore, ce sont les études qui viennent d'être faites sur la rubéole qui nous éclairent.

De même que pour la rougeole, on croyait que cette immunité était parfaite et solide, or nous savons maintenant qu'il y a énormément de réinfections. Ces réinfections se traduisent par une montée des anticorps et nous avons bien pu le voir chez les femmes enceintes. Or maintenant, nous disposons de très bonnes techniques.

Dans toutes ces infections virales, nous savons que lors de l'infection primaire, les anticorps spécifiques apparaissent d'abord dans la fraction Ig M,

les fractions lourdes des globulines, puis après dans les fractions Ig G. Au cours des réinfections, il n'y a jamais apparition d'anticorps dans la fraction Ig M, la montée des anticorps est seulement dans la fraction Ig G. Et déjà dans la rubéole, nous avons pu montrer très clairement que chez les femmes qui en début de grossesse avaient une ascension d'anticorps, quand cette ascension d'anticorps était seulement dans la fraction Ig G, il n'y avait d'abord jamais de virémie et les enfants ne sont jamais atteints par le virus.

Ce qui fait que nous disposons maintenant de techniques qui nous permettent, sur un seul échantillon de sérum de faire le diagnostic d'une infection récente. Une des plus belles applications de cette technique est faite dans un autre ordre de maladies infectieuses, dans la toxoplasmose qui est aussi un autre problème d'atteinte de l'embryon, cette fois par un parasite.

Les méthodes de Remington à Palo Alto et les méthodes de Desmonts en France, par la recherche systématique des anticorps antixoplasmose dans la fraction Ig M, recherche qui est faite par fluorescence, peuvent en examens systématiques — et cela est fait dans de nombreuses maternités à Paris maintenant — déceler les infections précoces par le toxoplasme et le déceler sur le premier prélèvement de sang, et là c'est très important, permettent une thérapeutique immédiate. Car on estime qu'il y a un délai entre l'infection de la mère et l'atteinte de l'embryon, délai qui est le passage à l'embryon, qui là n'est pas par voie sanguine mais qui est par passage trasplacentaire. Et actuellement, ces techniques-là permettent le traitement systématique de ces femmes, qui donc représente un traitement préventif de l'atteinte de l'embryon. Dans ce domaine des malformations congénitales, c'est la première fois qu'on a une attitude thérapeutique préventive grâce à un dépistage rapide de l'infection, grâce à ces dosages dans les Ig M. Donc voilà, à mon avis, une voie d'avenir qui s'ouvre et qu'il va falloir approfondir pour de nombreuses maladies virales.

Enfin dans un domaine plus fondamental, les études sur les cellules embryonnaires humaines ont apporté des renseignements très importants sur les infections virales tératogènes.

La première notion, vous le savez, a été la notion d'infection chronique qu'on a retrouvée pour la rubéole, qu'on retrouve pour les cytomégalovirus, puisque l'enfant infecté tôt dans la grossesse excrète encore le virus à sa naissance dans les semaines, les mois, voire les années après sa naissance.

Une autre notion a été aussi grâce au système cellulaire embryonnaire humain la démonstration de l'action du virus de la rubéole sur la division cellulaire, qui montre que selon le type de cellule, le tissu auquel elles appartiennent, selon leur état de différenciation, le virus peut inhiber sélectivement la division cellulaire ou entraîner seulement un ralentissement de la croissance de la population cellulaire qui explique ainsi le petit poids de naissance des enfants.

Nous avons donc dans ce domaine des voies qui s'ouvrent. Je crois que l'impression que nous avions il y a encore peu de temps sur le côté décevant du travail de la recherche des infections virales se trouve modifiée maintenant, grâce en particulier aux techniques basées sur la sérologie dans les fractions de globulines; ce chapitre est donc loin d'être clos, et au contraire s'ouvre et je crois que c'est une voie absolument prometteuse pour l'avenir.

DISCUSSIONS

K. H. DEGENHARDT : I have a comment and a question :

What are the experiences in France concerning the rate of pregnant women without antibodies against rubella, cytomegalia or mumps virus; what are the conversion rates, i.e. no antibodies in the first trimester, but antibodies on the Igm level in the second trimester as a proof of early virus infection.

From our prospective study we have some preliminary data on this, which were gained by Professor Haas in Freiburg. They are as follows : it seems to be that early virus infections in pregnancy are very harmful in the case of virus infection epidemies, but less in the case of single virus infections concerning the embryo.

A. BOUÉ : I don't have any experience of that but the result from the United States of the congenital rubella syndromes after the epidemic of 63-64 and now in the cases observed in the last years, were not very different regarding the frequency of congenital malformations. One of the problems is, there are now very few cases of congenital rubella, because we have serological diagnosis and most of the cases are aborted and the only thing I know is that when you have a true primary infection, with rubella virus in the beginning of the first trimester of pregnancy, and when you do virus isolation in the embryo, in 91 %, virus is recovered from the embryo. It seems now that congenital malformations caused by cytomegalovirus are numerically more important than those caused by rubella virus, but the problem is that in all the infections with cytomegalovirus, it seems that important congenital malformations are seen at birth in only few cases and we have to follow these children a long time to detect all the symptoms. This is the same with toxoplasmoses, you have to follow the child at least ten years to see the whole problem of the malformations from this infection.

J. EBERT : Did I understand correctly that you have speculated that rubella virus affects the rate of cell division ? Do you mean to imply a slowing (stretching out) of the cell cycle, or merely, that as a consequence of cell death or severe cellular anomalies, a number of cells are removed from the dividing population ?

A. BOUÉ : That is not exactly what we observed. You know that one of the main symptoms of the rubella syndrome is the low birth weight. We have done *in vitro* studies on human fibroblastic cell-lines, in the cell-lines initiated from

lung or from kidney and inoculated with rubella virus, a complete inhibition of cell division is observed and it is impossible to propagate the cells.

When the same experiment is done with cell-lines established from muscle and skin, it is possible to carry this cell-line chronically infected with rubella virus, and a high percentage of chromosomal breaks is observed. That does not mean that rubella virus induces chromosomal aberrations; a chromosomal break in one mitosis means that the daughter cells will not divide again. So in each replication of the cell population, a percentage of cells are unable to divide again, and this results in a slower increase of the cell population. Very recently in Nashville, Tennessee, there was a paper with the same model, inoculating not a virus but a drug — salicylate — the inhibition of the cell division is high in the kidney cells, in the lung cells, and there is no inhibition of the cell division when the cell-line is established from muscle or from skin. So there is a correlation between the cell differentiation and the sensitivity of the cells to an effect like a virus or like a drug.

J. EBERT : But all of these cells are fibroblastic in appearance ?

A. BOUÉ : Yes, except kidney cells.

J. EBERT : They are not producing product of origin; there are no muscle proteins produced in the muscle cell-line, am I correct ? These are fibroblastic cells ?

A. BOUÉ : Yes.

M. WINICK : Data in Rubella using manometric measurements indicate that the fetal organs contain a *reduced* number of cells and that the average size of the individual cell is also reduced.

K. HIRSCHHORN : Since the human fetus is apparently immunocompetent in later pregnancy, do we have two rubella syndromes :

1) infection in the first trimester with congenital anomalies and persistent viremia;

2) Later infection resulting in an autoimmune — like syndrome.

R. GOOD : The immunological events relating to rubella infection of the fetus are extremely complex. I could not at this juncture divide the influences of infection into those which occur early and those which occur late. In contradiction to earlier predictions of Burnett and others that early exposure to viruses or early infection tolerance has not been observed to any viruses of

which I am aware, indeed even fetuses infected very early with rubella virus will produce antibodies against the virus. When the rubella virus infections are persistent these antibodies are selectively Ig M and very little or no Ig G antibodies are produced. Indeed in such infected fetuses and those in which infection persists into the neo-natal period, Ig G immunoglobulin may be significantly inhibited and the children may have a immuno-electrophoretic pattern that would be interpreted as dysgammaglobulinaemia is frequent. With cessation of the infection and diminution of virus secretion one sees a return of the immune response to normal. I cannot conceive however, that the host forming antibodies and the virus being produced and entering the blood stream would not result in some degree of immune assault on the babies tissues as is observed drammatically with other know forms of persistent virus infections. Such assault might be if you will related to a kind of pseudo-autoimmune process.

C. LEVINTHAL : Does your data distinguish between the hypothesis that the increase in trisomy — 21 with age of mother is due :

1) to increase in number of trisomies at conception, or

2) to an increase of the percent of the trisomies which reach full term.

A. BOUÉ : Until recently, it was very difficult to distinguish between 21 from 22; but now with the new technique of fluorescence, it is easy to differentiate the chromosome 21 from the chromosome 22. Cell-lines were established from some of the embryos frozen and stored in nitrogen liquide. They were analyzed again with fluorescence and more trisomy 21's than trisomy 22's were found. In all the recent specimens we received in the laboratory in which we systematically do fluorescence to differentiate 21 and 22, we have a great number of trisomy 21.

When you do the curve of the frequency of the trisomy by maternal age, you have a curve like this one (drawing at the board). This curve is the same for trisomy at birth. This curve is bimodal with a peak at 27 and a peak at around 40 which can give two curves. The curve with the peak at 27 is exactly the curve of the normal births regarding the age of the mother. But this curve is also the curve of monosomy X, and the curve of the triploid specimens. For the trisomic conceptuses there are two curves : on which is not related to the age of the mother, and one which is dependent on the age of the mother in which there are mainly trisomy D and G.

J. G. BOUÉ : And now it seems that for one trisomy G 21 coming at birth there are around five abortions with exactly the same trisomy 21.

R. GOOD : It is of special interest in this regard that when the rubella virus or

the closely related Newcastle disease virus infect human lymphocytes, these agents can interfere in a dramatic way with the blast transformation induced by PHA or other mitogens allogeneic cells, or antigens to which host has been exposed. These findings indicate that, further, rubella virus has the potentiality to perturbe in a most significant way the immunologic functions of developing child. Perhaps the most extreme of such influence is the production of agammaglobulinaemia as one of the developmental consequences of this kind of virus infection.

To understand the Haas data which Dr Degenhardt mentioned, I would be most interested in knowing whether the evidence of conversion to Rubella, inclusion body virus and mumps virus reflected Ig M or Ig G antibodies or both.

R. TURPIN : Personne d'autre ne demande la parole ? Avant de donner la parole à Monsieur Hirshhorn, j'ai le plaisir de saluer la présence parmi nous de Madame Farçat et de Madame le Docteur Lefevre-Paul représentant, la première, Monsieur le Ministre de la Santé Publique Monsieur Boulin et Madame Lefèvre Paul, Mademoiselle Dienesch, Secrétaire d'Etat.

PRENATAL DIAGNOSIS OF EMBRYOPATHIES

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Some of the first successful attempts at amniocentesis were for the purpose of prenatal diagnosis of sex. This was shown to be possible by Sachs' group in Israel in the early 1950's, and was used for genetic purposes by Fuchs in Denmark a few years later. Fuchs performed the procedure on pregnant women at risk for having a boy affected with a sex-linked genetic disease, such as hemophilia or muscular dystrophy, and aborted all males.

At that time, amniocentesis was performed transvaginally, resulting in a number of infections. With the large experience, in the 1960's, in transabdominal amniocentesis late in pregnancy for the monitoring of cases of Rh incompability, this approach has now become standard for prenatal genetic diagnosis between the twelfth and eighteenth post-menstrual week.

From 5 to 20 ml of fluid is removed through a spinal needle, inserted under sterile precautions with trochar in place and without local anesthesia. The cells are separated by centrifugation, and the cell-free fluid may be used for certain biochemical studies, as is done in Rh monitoring. At our present state of ignorance as to the exact source and composition of the fluid, it is best to rely on the cells for definitive genetic studies.

Some studies are possible on the fresh cells without growing these in tissue culture. The recent discovery that a large segment of the long arm of the Y chromosome fluoresces brightly when stained with quinacrine, has made it possible to detect the presence of the Y chromosome in cells from males. Even here, however, there is some risk of error due to the presence in the population of short Y chromosomes which do not fluoresce, but which are not associated with disease. We, therefore, strongly suggest that sex diagnosis be based on actual chromosome analysis of cultured cells. It has been shown that a number of enzymes relevant for genetic disease can be detected in uncultured cells. Until now, however, very few of these have been shown to be practical measures for specific diseases.

It is, therefore obvious that the method of choice for prenatal diagnosis is

dependent on the ability of the cells from amniotic fluid to grow in tissue culture. Initially one sees cells of varying morphology. Within a day these settle to the surface of the culture vessel, and within a week a monolayer of fibroblasts can be seen. Soon thereafter, the cells can be harvested and split for further growth. At the next harvest, one of the bottles can be treated for chromosome studies or for biochemical analysis. Many more enzymes can be detected in cultured cells than in uncultured ones. New assays are added almost every week. The number of enzymes detectable in cultured skin fibroblasts is even greater, and it is likely that most of these will be detectable in amniotic fluid cell cultures. Several inborn errors of metabolism have already been diagnosed *in utero* by these methods, and the affected foetuses have been aborted.

The earlier finding that the diagnosis of Pompe's disease can be made from the amniotic fluid has now been shown not to be possible and this is one of the big dangers in the biochemical studies of prenatal diagnoses. The glucosidase that is found in the amniotic fluid, as has been recently shown by Nadler, turns out not to be the same glucosidase which is deficient in Pompe's disease. In other words, one must always be certain that one is not dealing with a isozyme of the particular enzyme deficient in a particular disease. Another error, as is now clear, deals with the previous of cystic fibrosis and Marfan's syndrome by means of metachromatic granules in the cultured cells from the pregnancy. This is first of all a highly non specific finding, now having been demonstrated in some 25 different diseases, and secondly even in cases of cystic fibrosis where metachromasia is found in the fibroblasts of affected individuals, it can be absent in the amniotic fluid cells.

The other diagnoses that have been made have been confirmed on the tissues of the abortuses.

Because of the high frequency of chromosome defects, and because of the relative ease of the technique of chromosome analysis, most of the cases studied have been in the field of cytogenetics. The vast majority of these studies have been for the diagnosis of Down's syndrome or mongolism, associated with trisomy of chromosome number 21. Mothers over 40 are at increasingly high risk for having such children, and younger mothers who have borne a child with trisomy 21 also have an increased risk of recurrence. Even greater is the risk of recurrence in a mother who has borne a mongoloid child carrying a centric fusion type of translocation, as, for example, between a 21 and a 15 chromosome.

This is of course only true if one of the parents particularly the mother, carries the translocation in a balanced state. Such translocations are initially the result of two breaks on opposite sides of the centromeres of the two relevant chromosomes. When the broken ends join, a translocation chromosome is form-

ed. The smaller product is usually lost. At meiosis, the translocation chromosome pairs with the two homologous normal chromosomes. First meiotic division can then result in a variety of gametes, the most important of which are respectively normal, balanced, carrying the translocation chromosome, and unbalanced, carrying the translocation chromosome and one of the two normal ones. The zygotes produced after fertilization will be, respectively normal, clinically normal translocation carriers at high risk for abnormal offspring, and trisomies with the extra chromosome attached.

Actual results have shown that the risk in carrier mothers is about one in three of having an affected child, and the same for producing a carrier like herself. This risk of recurrence is higher than that previously estimated from retrospective studies, and has been confirmed in additional families. These data represent a collection of about six months ago from four laboratories in the U.S.A., those of Dr. Nadler, Dr Valenti, Dr Jacobson and ours.

A high risk must also be expected in cases of reciprocal translocations, found in a number of families with children with congenital anomalies or multiple spontaneous abortions. These result from breaks in two chromosomes and reciprocal translocation of the fragments in one of the ancestors. During meiosis the two translocation chromosomes pair with the two normal homologues, and first meiotic division results again in normal, balanced and unbalanced gametes, which, after fertilization, will produce zygotes which are normal, clinically normal balanced translocation carriers at high risk for abnormal offspring, and unbalanced offspring with duplication of some chromosomal material and deficiency of other parts, resulting in either foetal death or a liveborn with anomalies. It is obvious that such families will greatly benefit from prenatal chromosome studies.

In the combined experience of six laboratories in the United States, as of the beginning of 1971, the vast majority of indications for amniocentesis have been for chromosome analysis. Of these, the two largest groups have been mothers over 40 and mothers who have previously given birth to a child with trisomy 21. A smaller number were translocation carriers and another small group had other indications for chromosome analysis, such as radiation exposure. A moderate number of pregnancies were monitored for diagnosis of sex, because the mother was at 25 % risk for having a boy with a sex-linked disorder. The remainder were studied for a variety of inborn errors of metabolism.

The results of these cases show the high risk in translocation carriers about 1 in 3, a higher than expected risk of mongolism in children of older mothers, that is, about 4 %, and also a higher risk than previously calculated for recurrence of mongolism in younger mothers who have produced a child with

trisomy 21, that is about 3 %. The other results fit the expectations reasonably well.

As you can see much has already been achieved in the field of antepartum diagnosis of genetic defects.

I think that some words should be said about the problems that remain. First and much talked about, is the ethical problem. I will leave that simply by stating this is something for the physician and the parents to work out.

The second deals with the question of risks. In approximately one thousand cases now studied in the United States, there has been no case of maternal mortality due to this procedure. So the risk to the mother is smaller than one in a thousand. In terms of the foetus, one can conceive of three types of risks : first, death of the foetus by abortion. In approximately 700 cases sufficiently studied for this purpose, there have been 3 abortions found, 2 or these however, occurred longer than two months after the procedure and it is doubtful whether these were related to the procedure. The third occurred approximately two weeks after the procedure and one does not know. The rate of spontaneous abortion in the second and third trimester expected in 700 pregnancies is certainly no lower than this.

The second problem would be congenital malformations or injuries to the foetus. One would not really expect congenital malformations because the organs of the foetus are already formed at this particular time. Injury to the foetus has not been observed in the over 600 children that have been delivered following this procedure.

Now comes the question of long-term risk to the foetus. It has been suggested by some that the removal of amniotic fluid may result in temporary hypoxia to the foetus and this could conceivably cause brain damage. There is now a study under way in the United States to attempt one and two year follow-up of children born following mid-trimester amniocentesis and some control children in order to evaluate this possible but to me somewhat unlikely risk.

The third question deals with what can be done with this gold mine of information, the amniotic fluid. Our ignorance in this field is enormous. We still do not know at any particular time of pregnancy what comes from the foetus and what comes from the mother with the exception of very few components and even those have not been thoroughly studied. For example, we have recently found, together with Dr Ainbender at Mount Sinai that in mid-trimester, we can find maternal Ig A and Ig M antipolio antibodies in the amniotic fluid. This, of course, might play a large role in protection against viruses that enter by means of the gastro-intestinal track as opposed to Ig G which enters the foetal blood.

The question of using amniotic fluid for genetic diagnosis is beginning to be approached but certainly is nowhere near a practical end.

The next problem is that each biochemical diagnosis needs a major research study in order to find out what the normal values are for the enzymes, whether it is the same enzyme as one is interested in or a different isozyme and a whole variety of other problems. We need to increase the number of diagnoses we can make, we need to worry about the problem of what can we do to diagnose those inborn errors, in which the normal enzyme cannot be detected in amniotic fluid cells. One possible approach that is now being worked on, is the possibility of induction of enzymes in tissue culture cells by a variety of techniques. Some help can be gotten perhaps from thorough genetic linkage analysis for the diagnosis of dominant diseases, and this is already possible in appropriate families with myotonic dystrophy since this gene is closely linked to the secretor gene for ABO blood groups, which can be detected in the amniotic material.

The next question is visualization of the foetus. We can now see some parts of the foetus by ultrasound; we can define quite closely certain external features of the foetus by foetography, by injecting a lipid soluble dye into the amniotic fluid at about 19 to 20 weeks of pregnancy which goes into the lipid material which coats the skin of the foetus, and then by an X-ray demonstrate the head, the back, the genitals and the extremities of the foetus quite well, so that diagnoses of anencephaly, meningomyelocele and a few others have already made by this technique. Scrimgeour in Edinburgh has recently perfected a foetoscope with which he has now examined four pregnancies at high risk for congenital malformations of which three were found to be normal and two have then gone on to normal delivery. So there is great hope for direct visualization.

When one visualizes the foetus, one can beautifully see in Scrimgeour's pictures the placental vessels and the foetal vessels, so the possibility of blood samples from the foestus becomes real for the diagnosis perhaps of certain other diseases. In fact, Arthur Silverstein at Johns Hopkins has succeeded with lambs and with monkeys to partially externalize a foetus for the purpose of blood sampling and, of course, as the most exciting prospect, for the potential purpose of therapy of a variety of disorders. It is this last situation, of course, that is our eventual aim as physicians. If we can diagnose diseases early in uterine life and if we can conceive of a therapy, this may avoid the final ethical issue of abortion and the final ethical issue of the true role of the physician.

I believe that it is safe to predict that, in the most too distant future, the great majority of pregnancies at risk for genetic or congenital disease will be amenable to prenatal monitoring. This should result in a marked reducting of the number of tragic events leading to incalculable emotional, social and economic cost.

ANALYSIS OF GENETICALLY DETERMINED ABORTIONS (*)

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The collection of unselected spontaneous abortions seems to be appropriate to elucidate the etiopathogenesis of intrauterine mortality. This communication is a first preliminary report on observations, which were made in a series of spontaneous abortions. They occurred in a prospective longitudinal investigation, which was initiated in the year 1963 by a committee of the German research foundation. The aim of the whole project was to intensify research about the etiology of congenital malformations and further on to elucidate the causes of prenatal losses, perinatal mortality and morbidity. Gravidas were registered as early as possible in the first trimester of pregnancy. Up to October 1971 there were 1.181 abortions documented. This means a rate of 9.3 % abortions related to 12.698 gravidas. Approximately 20 % of all spontaneous abortions gave materials, which could be sent to the 10 collaborating cytogeneticists. However, experiences were made, that only 50-60 % of these abortion materials could be successfully cultured for chromosomal analysis. Our observations concern 127 spontaneous abortions cytogenetically defined.

TABLE 1

Spontaneous abortions.

time of abortion (weeks p.m.)	Karyotype		total
	normal	abnormal	
$\leqslant 13$	28 (56 %)	22 (44 %) (*)	50 (39 %)
> 13	64 (83 %)	13 (17 %) (*)	77 (61 %)
total	92 (72 %)	35 (28 %)	127 (100 %)

(*) Significance 0.1 %.

(*) Vortrag a.d. Fourth International Congress of Human Genetics, Paris 6. 11.9.1971. und a.d. Symposium l'Institut de la Vie, Château de la Verrière, 2. 4.12.1971.

Table 1 presents a summary of the rates of chromosomal anomalies in 50 early abortions and 77 late abortions. The end of 13 weeks after the last menstrual period was the limit for this subdivision. There is a remarkable difference between the rate of chromosomal anomalies between the two groups of early and late abortions. The relative high rate of chromosomal anomalies in early abortions might be considered as a minimum rate. The sex ratio (*) in early abortions with a normal karyotype was 4 : 24 males : females; this rate differs from all other groups. We suppose, that at least in some cases maternal

TABLE 2

Sex ratio in spontaneous abortions.

Karyotype	Early AB. n		Late AB. n	Σ
Normal	♀	24	40	92
	♂	4	24	
	♀	16	6	35
	♂	6	7	
		50	77	127

TABLE 3

Spontaneous abortions; chromosomal aberrations.

Types	*n*	%
45, X	8	22.9
Trisomy A	2	5.7
(2)	(1)	
(3)	(1)	
B	-	-
C	2	5.7
D	3	8.6
E	7	20.0
(16)	(6)	
(17-18)	(1)	
F	-	-
G	6	17.1
Double Trisomy	2	5.7
Triploidy	2	5.7
Others	3	8.6
Total	35	100.0

(*) Table 2.

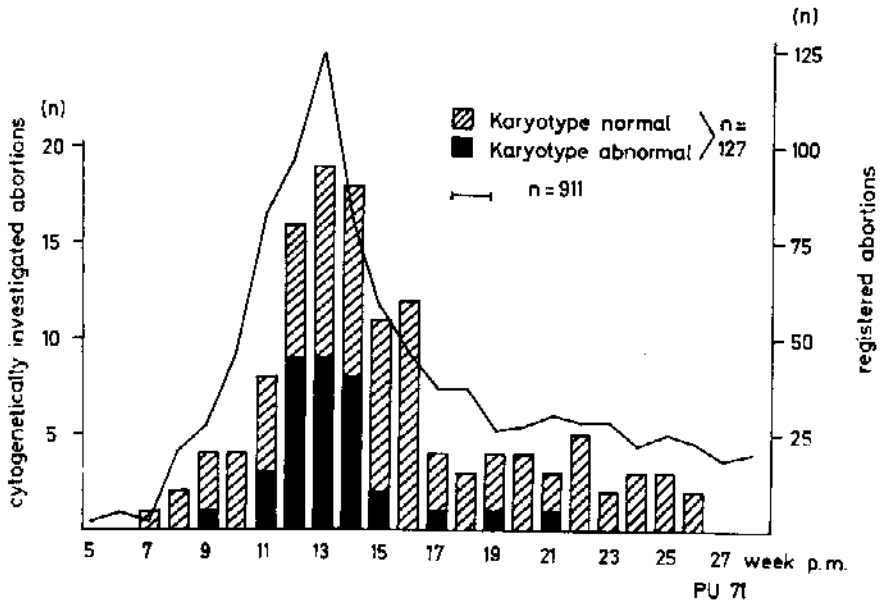
tissues may have been cultured. This might lead to an underreport of chromosomal aberrations in early abortions. The different types of chromosomal aberrations are summarized in table 3. Trisomies are seen most with a frequency of 62,8 % of the 35 chromosomal anomalies. The differences in the rates of chromosomal anomalies between early and late abortions are effected by the frequencies of trisomies, which are 4 times higher in early abortions. Two cases of double trisomies were seen, i.e. 48, XXA2$^+$, C$^+$ and 48, X, C$^+$, E16$^+$. The larger the chromosome involved, the less it is encountered in trisomic form. This observation of many cytogeneticists may be corroborated here and also the notable exception in group F chromosomes.

XO monosomies account for 22.9 %; this is in the expected rate. There seems to be an underreported rate of triploidies. In one of two cases a diploid/triploid 5 : 95 mosaicism was found with the aberrant cell line 69, XXY. This abortion ocurred 19 weeks after the last menstrual period. This type of mosaicism is relatively rare among abortuses. In this cases only egg membranes and placental villi had been received for histological analysis. The other case was a pure triploidy with 69, XXY. This abortion ocurred 14 weeks after the last menstrual period. A ruptured sac with a cord present but no embryo was sent for histological analysis. The term "Others" in the table means structural aberrations, i.e. 2 times prolonged arms of one chromosome 16,1 mosaicism with a D/G translocation cell line.

We raised the question : "What may be the importance of self-selection in this series of 127 spontaneous abortion ?" Our test on this subject may be encouraging. Table 4 demonstrates the distribution of the 127 abortions with regard to the weeks of pregnancies, when they occurred. Abortuses with chromosomal aberrations are easily recognizable. There is a clear peak of frequency between 12 and 14 weeks of pregnancy. We made a comparison with the distribution of 911 spontaneous abortions from which only about 14 % were analyzed cytogenetically. The peak of frequency coincides with the peak of the 127 cytogenetically defined abortions. This observation might be interpreted in the sense, that selection of the abortions materials for chromosomal analysis was by chance and did not influence the spectrum of chromosomal aberrations. Furthermore, we noticed, that abortions with chromosomal aberrations occurred three weeks earlier in the mean than those with normal karyotypes.

Hispathological examinations of the abortion materials were performed by local pathologists in close cooperation with the 18 collaborating clinics of gynecology and obstetrics. During the last 3 years two centers were active in this respect, one at Berlin with Prof. K. F. Kloos and the other in Bonn University with Dr Kühl. Evaluations and comparisons between histological and cytogenetical findings have not yet been finished. I shall restrict my report to a few general remarks.

TABLE 4
Spontaneous Abortions.



Prof. Kloos focussed his attention in our collaboration project exclusively to histological examinations of early abortions and made very thorough documentations. For this he developed a new extensive scheme. His impression up to now might be summarized as follows : He could not yet corroborate the findings of associations between specific types of chromosomal aberrations and distinct signs of placental anomalies in the sense of disturbances of formation or differenciation. However, we should acknowledge this with great reserve until the evaluations have been finished.

DISCUSSIONS

M. WINICK : Amniotic fluid non-dialyzable hydroxyproline concentration is linear to 28 weeks and then drops. We are hopeful that amniotic fluid AMN Hydroxyproline concentration may be a marker of fetal growth.

AA. MOSCONA : Is it known in some detail what type of cells are present in the amniotic fluid ? It is important to be able to distinguish not only between fetal and maternal cells but also between the different kinds of fetal cells present in the fluid at different stages of fetal development.

Have methods of differential cell fractionation and cell cloning been applied to these cells; such approaches would be essential if one were to use these cells in a meaningful system of screening for biochemical abnormalities, for enzyme surveys, etc., in these cells. Primary cultures of these cell populations as they seem to be used now, are of limites analytical usefulness, because of the possible initial complexity of these cell populations and because of the secondary cell interactions and cell-medium interactions in the evolution of such cultures, and the selective events that might occur in it.

Finally, the cell population in the amniotic fluid may be of a "selected" kind due to the particular properties of this fluid and of the amniotic environment. The exploration of these and related problems of this is practically feasible, should help to clarify the eventual usefulness of amniocentesis as a diagnostic tool.

K. HIRSCHHORN : With reference to the first question, there is really only one definitive study until this time and that is by Nadler, recently published, in which he has demonstrated that if the amniotic fluid cell culture results in a monolayer of epithelioid cells as opposed to the more frequently observed fibroblastic type of out-growth, there are definitely differences and the one that he has chosen to study is the enzyme histidase which is deficient in the disease histidinaemia. He has been able to show that in normal amniotic cell cultures of the epithelioid type, this enzyme is present, while in the fibroblastic type it is absent.

Now generally, in most people's experience, the cells will grow out either as fibroblasts or as epithelioid cells and only rarely ever as a mixture. As to contamination by maternal cells, this has been now checked by a variety of techniques including one that I would suggest to Dr Degenhardt to distinguish

maternal cells. This is by means of enzyme polymorphisms known to exist in the mother and looking for heterozygosity in terms of paternal contribution to distinguish foetal from maternal cells. It has also been shown that if a culture of amniotic fluid cells grows very rapidly, so that one has a fibroblast layer in three to four days, it is almost a *sine qua non* that this is maternal tissue.

Even if this happens and one lets this culture grow on or, as we do now, has multiple bottles and takes the more slowly growing tissues, they are invariably, at least in those that have been studied until now, foetal. So the kinetics of cell growth between maternal and foetal cells is quite different and I don't think that this will be a great problem in the future.

Fibroblasts are not all the same. One can show, for example, as we have in a recently published article, that fibroblasts established from bone marrow are most likely hemopoietic stem cells as opposed to fibroblasts grown from skin biopsis. So the appearance of fibroblasts tells you absolutely nothing of their source or of their potential. Therefore, your suggestion of cloning out these cells is a good one. However, there is another finding which I should mention which is that in one bottle of amniotic fluid cells, it is very likely that cloning already exists; in other words, if one observes these cultures, one finds most frequently one point in the bottle that begins an outgrowth and that is what then eventually covers the whole bottle.

We even have, as an example of the kinds of problems that can arise with amniotic fluid cell diagnosis, a case that is, I think, rather crucial : a fluid was sent to us for the potential diagnosis of Tay-Sachs disease derived from a mother who had previously had an affected child. We grew the cells and did the enzyme analysis for hexosaminidase A and concluded that the child did not have Tay-Sachs disease. The mother was 36 years old and an intelligent psychiatric social worker and requested from the original laboratory that had sent us the sample that we look at the chromosomes of the cells that were grown. We agreed to do this and found 45 chromosomes in every cell. Ordinary analysis would have told us that the most likely diagnosis was 45 XO Turner's syndrome. We did note, however, a number 13 chromosome which had extra material on its short arms. We then performed fluorescence analysis by the quinacrine technique and found that every interphase cell had the fluorescent Y body. We could not find the Y initially in the karyotype but knew that it had to be there somewhere, at least the long arm of the Y, which is all fluoresces but does not contain the masculinizing genes. Fluorescent analysis of the chromosomes demonstrated that the extra material on the chromosome 13 fluoresced in the manner of a long arm of a Y chromosome. We brought in the family to tell them of these findings. We explained to them that we could not predict the sexual development of this fœtus, that it could be a Turner's syndrome, a normal male or an intersex, and they elected to abort on this

information, already having one normal and one grossly abnormal child with Tay-Sachs diseases. At the time of abortion which was done by hypertonic saline, a fresh sample of amniotic fluid was obtained. Growth of that sample demonstrated all the cells in several bottles to be 46 XY. Regrowth of a sample that had been laid down from the initial culture that had demonstrated 45 chromosomes, continued to show 45 with the Y, 13 translocation. We are quite convinced that this was a chance clonal development of an aberrant cell line, either developing in culture or in the embryo itself.

With reference to your second question, of amniotic fluid as a culture medium, Maimon Cohen in Buffalo routinely cultures his amniotic fluid cells in amniotic fluid until they become established. We have studied this side by side with conventional media; we happen to use McCoy's medium 5 A with 30 % foetal calf serum, and we found no difference between the two methods in efficiency or in the biochemistry as far as we have studied it, or in the chromosomes complements.

D. HSIA : I wish to emphasize that the biochemical analysis of fetal cells is a different order of magnitude from cytogenetic problems. By biochemical problems, one meant :

1) establish normal enzyme and isoenzyme patterns,
2) determine biochemical abnormality — the homozygote,
3) distinguish variant,
4) detect the heterozygotes.

A. E. HELLEGERS : I did not see Dr. Winick's slide on hydroxyproline concentrations long enough to see whether the decrease in the amniotic fluid concentration parallels the decreasing osmolarity of this fluid. Since the osmolarity decresaes from about 290 to 250 at term, I would like to see the slide again to find out whether increasingly hypotonic urine, rather than hydroxyproline metabolism could explain the data.

V. INGRAM : Abnormal karyotypes are observed in cell culture after many generation in culture. Does this accurately represent the karyotype of cells in the fetus, rather than an effect of culture conditions ?

K. HIRSCHHORN : In those cases in which it has been possible to obtain tissue from the embryo following abortion, when a chromosomal aberration has been detected in amniotic fluid cell culture, this has been identical, with the one exception of the case I mentioned previously. So I am not saying that the possibility of either selection of a preexisting aberrant clone, or of mosaicism, or of

some event occurring in tissue culture cannot happen. All that I am saying is that in the great majority of cases it seems to have not happened.

V. INGRAM : But that really does not explain the situation or does not provide an answer because presumably when you test the tissues of the aborted fœtus, you go through a similar procedure of culturing the cells.

K. HIRSCHHORN : Yes, but now what you are askink for is for the same chance event to occur in tissue culture to the tissue derived from the amniotic fluid cells *and* the tissue derived from the fœtus, and that these should produce now identical chance aberrations whereas in culturing hundreds of samples of amniotic fluid cells these have not occurred. It is asking I think for too much chance.

V. INGRAM : No, no, not a chance event, but the susceptibility, I am asking is the actual situation, not the production of abnormal caryotypes and therefore abnormal products in the fœtus, but rather the susceptibility of the cells to the environment which makes them react in this particular way, which would put the cause elsewhere ?

K. HIRSCHHORN : I think you can ask that about every cytogenetic study in existence except for direct chromosomal analysis of bone marrow samples.

V. INGRAM : Will Dr. Hirschorn's medical intervention *in utero* for abnormal fetuses to bring them to term result in an increase of deleterious genes in the population ?

K. HIRSCHHORN : The question of the genetic decline of the human population was raised in eugenic terms initially by Galton in the XIXth century and has been a recurring bogeyman raised by some individuals since then. For a whole host of reasons, I don't believe that this is a matter with which to concern ourselves in any worrysome manner. There are now several very good analyses for example, by Medawar, to why it is not a real problem. Let us get to specific situations.

In the case of a rare recessive gene, the maintenance of the individuals with the rare disease, if they propagate will cause a doubling of the gene frequency in about two to four hundred generations.

The rate of advance of medical science, I think, is several logs higher than this kind of doubling and we have invented glasses to allow you and me to cross the street without getting run over.

The question of a common recessive gene, such as Tay Sachs disease in the Jewish population, all that I can do for the moment, not wanting to get into a really prolonged debate about this, is to quote Harry Harris' comment from the recent International Congress of Human Genetics held in Paris in which exactly this question was asked. Does amniocentesis and abortion, selective abortion — it is really a variation of your question — of homozygotes not cause an increase in the gene frequency of the gene, because two out of the three children that will be born will, in fact, be heterozygotes and there may be reproductive compensation and so on, causing then a gradual increase and in the case of a common gene, a more rapid increase of the gene frequency ? Harris' answer to this was that if Ashkenazy Jews were to marry non-Jews, this would no longer be a common disease.

C. LEVINTHAL : I had several questions of which the one that Vernon Ingram just raised was one. One is a friendly question and one is an aggressive comment. So let me make my friendly question first and then the aggressive comment which I think that Dr. Hirschorn has probably heard before, and I know he will hear it again.

The friendly question is : in your envisaging the carrier situation, does that give you the possibility of asking the question what fraction of a particular trisomy gets through to term ?

And it seems to me it does and it seems to me that, at least, *grosso modo* the answer would be most of them; otherwise your statistics wouldn't come out at all right, but maybe I misinterpreted it. Maybe we had better forget that one for the moment.

Now my aggressive comment : safety of amniocentesis has not been adequately determined. The increase in use of the procedure should be carefully controlled and *all cases* in which the procedure is used reporting should be required and follow-ups on children should be done.

K. HIRSCHHORN : Let me start with the friendly questions : What you were talking about was the frequency of abnormals detected in the translocation carriers for D, G translocation mongols. And I am afraid that from a number of animal experiments, it is not *a priori* predictable what percentage of gametes will result in an unbalanced state. This is due to the fact of the manner in which chromosomes line up during meiosis, and the adjacent segregation which would result in the abnormal ones, in a variety of translocations in both plant and animal material, results in highly variable proportions of abnormal zypotes. Therefore, just because we have found one third of them coming through as abnormal does not mean that one third started out as abnormal, so I am afraid one cannot answer that.

Now let me answer the aggressive comment with an not quite so aggressive answer. First of all, the word " *perfectly safe* " was never used as far as I know in my, Henry Nadler's or anybody else's talk that I have ever heard on this topic. All that we can give at this particular point are minimum risk figures for differents kinds of events : maternal minimum risk figures, fœtal minimum risk figures for immediate damage, but we have no idea, as I said before, of fœtal minimum risk figures for long term damage, if any.

The question of who does the procedure, I think is enormously important and this ought to be remembered until we have either the knowledge that any obstetrician can do the procedure equally safely in terms of the mother and, in the long run after we have more data, in the fœtus, we must restrict this procedure to high risk cases. And when we now say high risk cases, the risk for the total population in whom this is done is not one or two in a thousand but for the vast majority of these, it is far higher. For a translocation carrier is one in three, for an inborn error of metabolism it is one in four, for a woman over forty it is three in a hundred, which is thirty in a thousand. It is not one or two in a thousand.

Therefore, I think that in these high risk situations, we are perfectly justified in accepting the current minimum risk figures as being considerably lower at least for what we can measure at this time than the immediate severe and frequently prolongedly lethal effect of what we are looking for. On the question of reporting, I completely agree with you and a number of us have, in fact, come together and are exchanging this information for the sake of reporting. The information is quite complete in terms of success of culture, in terms of a whole variety of questions. As to whether or not the government will require this reporting, personally I think it might be a rather good idea, but you also, I am sure, are well aware of the fact that not only physicians but most people in the United States are a bit leery of government's requiring the reporting of something and would rather do these things on a voluntary basis which I think will become very efficient and is already quite efficient, as you can see from the collaborative data that I have presented until now. A similar situation exists in the case of transplantation.

W. McBRIDE : I would like to ask Dr. Hirschorn, I am sorry I may have missed a little, did you mention you had one failure ? How many pregnancies have you terminated following amniocentesis ? Was this the only failure that you had, the only failure in your diagnosis in the Tay Sachs disease ?

K. HIRSCHHORN : The failure was not in Tay Sachs disease, the failure was a chromosomal problem. In our laboratory this happens to have been the only failure. We have terminated in our laboratory at this moment nine cases of which

eight have been confirmed for their diagnosis and the ninth was the case that was just talked about. I know that in Henry Nadler's study, there have been two problems in the very early stage of falling into the trap of studying maternal cells and the failure was quite the opposite, when an abnormal offspring was born with the diagnosis of normality. From what I can gather at this point, from the data in this collaborative study, the other cases have been confirmed if called abnormal. Now a few abnormals have been born despite a diagnosis *in utero,* but this has been because of one of two reasons : one, the study is done too late in the pregnancy to do anything about it, or there was a severe delay in arriving at a biochemical diagnosis, so that it became too late in the pregnancy, or second, maternal choice which amazingly enough to me has occurred in two cases that I know of, in which children that were diagnosed as abnormal, one a case of mongolism that I know of and another a biochemical error, I have forgotten the detail on that one, have been born at the choice of the mother.

W. McBRIDE : So, I think you said that you had done almost a thousand in the series, the combined series, and only nine pregnancies were terminated.

K. HIRSCHHORN : Nine in our laboratory, but the total number terminated was somewhere between thirty and forty out of the thousand.

S. BENNETT : We have learned that a high proportion of fetal abnormalities are accompanied by gross karyotypic abnormalities. The majority of genetic abnormalities are represented in karyotypes which appear normal by conventional methods. We should encourage search for methods which might pick up a greater proportion of abnormalities connected with genetic defects. Biochemical analyses of fetal cell cultures in search of phenotypic expression of enzymatic activity is too expensive and tedious for general use. Recent applications of fluorescent staining of chromosomes has revealed banding patterns on individual chromosomes, has facilitated the identification of individual chromosomes. We can expect refinements and advances in such techniques, and from such advances we can expect a broadening of the amount of genetic information which can be derived from study of mammalian chromosome smears. We can hope for an extension of the morphological information content of chromosome smears of mammals into as much detail as is revealed by the banding pattern of Drosophila chromosomes. Perhaps application of the electron microscope to study of mammalian will take us in this direction.

K. HIRSCHORN : I would like to clear up a couple of points. First, the particular banding techniques whether by fluorescence or by Giemsa banding in current

use, and by current I mean the last few months, have absolutely nothing to do with the bands observed in polytène chromosomes in drosophila; they are totally different kinds of things and I think the two should not even be mentioned in the same sentence, because there is a great deal of confusion between these two things just because superficially, they look somewhat alike.

Secondly, nucleotide change type of mutations cannot be detected even in the polytene chromosome of drosophila by a change in the striping pattern of that chromosome; it is deletions, duplications and other gross chromosomal anomalies — and by gross I mean beyond the gene level, that can be recognized. At this particular state of the art, I have very little hope that in the near future, we can get down to a nucleotide change level to pick up and screen for inborn errors of metabolism by any morphological technique that has until now been conceived.

K. DEGENHARDT : What do you think about non-disjunction in meiesis, at what stage it may happen preferently ? I would like to focuss your attention to the statement of Professor de Grouchy in Paris that non-disjunction leading to trisomy 21 could be related to the second meiotic division in oogenesis by a marker chromosome of the mother. Are there other indications that the second meiotic division may be involved more frequently ?

K. HIRSCHHORN : As you are probably aware in cases of sex chromosome aneuploidy, of the X chromosome particularly, where one can get genetic information through Xg — a blood group study, and determine whether the non-disjunction occurred paternally or maternally and whether in the first or second meiotic division, the majority have been second meiotic division non-disjunctions, but there have been cases of first meiotic division non-disjunctions. So both seem possible although the second seems more likely, at least for the X chromosome.

Now as to other causes of trisomy 21, in the group of mothers where there is no relationship to advanced maternal age, careful analysis of both parents form multiple tissues has already demonstrated a higher degree of mosaicism among the parents than had been expected. We have recently, for example, found this in three fathers of mongols born to young mothers; there have been previous studies in which several of the mothers have been found to have this. One cannot rely on blood culture alone for this diagnosis and I am sure that with multiple tissue analysis a certain proportion of the young mother cases of mongolism will be demonstrated to be due to parental mosaicism. In fact, Penrose had some data some time ago in which he showed that in young mother mongolism there was some increase in grand-maternal age which could indicate a non-disjunction producing a mongoloid mother or father who through anaphase lag became mosaic.

R. TURPIN : Si l'intérêt d'une séance se fonde sur sa durée, je me permettrai de vous faire remarquer que nous siégeons depuis près de quatre heures. Sans doute, des questions ont été posées qui anticipaient sur l'avenir. J'espère donc que dans un avenir proche, nous nous réunirons de nouveau pour réexaminer ensemble les intéressants problèmes qui ont été envisagés ce matin.

Journée du 3 décembre 1971

Seconde séance

STRATÉGIE SCIENTIFIQUE

PRESIDENT C. LEVINTHAL

C. LEVINTHAL
Chemical teratogens at low doses

Discussions

M. COHEN et A. ROBERTSON
Birth defects, developmental control, and the screening of teratogens

K. DEGENHARDT, V. BECKER, R. HASS, K. KNÖRR, S. KOLLER et H.R. WIEDEMANN
A multiregional prospective investigation on pregnancy course and child development; a first preliminary evaluation

A. MONROY
Oogenesis and malformations

Discussions

CHEMICAL TERATOGENS AT LOW DOSES

Cyrus LEVINTHAL
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I would like to discuss several questions both practical and theoretical which seem to me important in connection with attempts to understand and ultimately reduce the factors which produce major physical and mental birth defects. Before doing so however, I would like to present a summary of what I believe are the most important ideas we have heard so far at this meeting.

There are three general conclusions which seem to me to have been demonstrated in several papers. First, and most important, is the fact that the fraction of children born with major physical or mental defects is probably higher than 5 %. This large proportion includes mental as well as physical defects which may not be observed until the children are ready to start school, but the numbers seem to be comparable in studies done in several parts of the world. Second, and clearly related to the first, is that increased wealth in a society and improvements in medical care can increase the number of individuals who are living major defects. In wealthy societies the life expectancy of children born with mongolism has increased markedly in the last several decades. In addition, results presented here suggest that a very large fraction of the products of human conception carry chromosomal abnormalities which cause early spontaneous abortions. It seems likely that medical care which is currently available can increase the number of children born with such abnormalities by reducing the selection against fetuses carrying them. Thus it is possible that the " best " medical care which can be given to pregnant women and to newborn children might increase the number of individuals in the society who are unable to care for themselves and thus require that the society devote enormous emotional and financial resources to their care. The third general conclusion with which I have been impressed, is that we have no real understanding of what causes these defects. We do not know, even approximately, how many of them are induced by : (a) environmental conditions external to the mother; (b) environmental conditions which the mother produces for the fetus; (c) genetic factors contributed by the parents, or (d) genetic anomalies which arise in the offspring. In addition to

our ignorance of the relative number of individuals effected by each of these causes, we have almost total lack of understanding of the mechanisms which are responsible for the defects in each of these categories. In spite of the fact that we don't know the relative proportions of defects caused by each of these possible causes, I will raise questions which relate primarily to the first and second. In particular, I would like to discuss in general terms the question of what the society can do in attempting to reduce the number of defective children whose defects might be caused by specific chemical substances which the mother ingests before or during pregnancy.

In considering the problem of chemical teratogens there are two quite different levels which must concern us. The first is that characterized by the thalidomide disaster in which compounds are ingested at high concentrations, that is, concentrations which are designed to produce pharmacological effects of some kind. In this case one is concerned with the same general problem which has engaged the attention of teratologists for many years. How can drugs be tested to be sure that they do not have unanticipated side effects on the fetus if they are administered to a pregnant woman. The detrimental effects, if they exist, would be expected to produce damage in a large fraction of the population exposed at a sensitive period of development.

The second level at which one must be concerned with the ingestion of chemical compounds by pregnant women is that which occurs when the material is consumed at a very low concentration. In this case, one might be dealing with materials which are not designed to be ingested by man but which enter the food chain where they are concentrated by various natural processes. Whatever the route of entery into the food supply, the concentration of possible toxic materials ingested by pregnant women would presumably be so low one would not expect a dramatic effect in which a large fraction of the exposed population suffered damage. In this case no direct observation of individual children with specific defects could be observed. If there were an effect, it would be a statistical increase in the number of birth defects but the increase might be so small as not to be detectable above the spontaneous rate. It is important to determine if such an effect is being induced by chemicals in the environment even if increase in the rate were small since the absolute number of individuals being affected could be very large even if the increase were not directly observable.

In fact the society frequently operates with the assumption that compounds which show teratogenic effects of high doses also do at low doses in spite of the rather anti-theoretical attitude on the part of some terotalogists who express the view that if they cannot observe an effect, it does not exist. The same argument has gone on for many years between many geneticists who have advocated reduction of radiation exposure on the basis of theoretical

extrapolations and many radiologists who have required direct observable evidence of ill effect before accepting limitations on diagnostic radiation exposure.

This issue has recently been faced by the American military when it was made to stop the use of the herbicide 2-4-5-T after laboratory tests showed that commercial preparation induced birth defects in experimental animals. The use was stopped even though it was claimed that the concentrations to which human populations were exposed were somewhat lower than those that had been used on the experimental animals.

In making decisions of this type one must make some implicit or explicit assumption as to the shape of the dose-effect curve, otherwise there is no basis for extrapolating from experimental results to real situations whenever any decisions or public policy must be made.

The Dose-Effect Relationship.

Measuring the effects on a population of a graded series of dosages of a chemical is a general method that may be used to detect dangerous substances. The " dose-effect " relationship is conceptually very simple and has proven to be a very powerful analytical tool. For example, dose-effect experiments have been used to show that *no* amount of ionizing radiation is too small to cause mutations. They have been used to show that some chemicals may induce cancer when applied, even in very low doses, whereas other chemical are carcinogenic only above a critical threshold level.

There are two fundamentally different idealized types of dose-effect relationships. In one type, the observed effect — be it cell death, irritation, number of birth defects, mutations, etc. — increases in proportion to the dose when the dose is very low. Some response is evoked at every dosage above zero. The practical significance of such a low dose relationship is that no matter how little there is, or how dilute, the agent poses a calculable threat. For instance, the carcinogenicity of some chemicals is described by a linear dose-effect curve. A given amount of one of these chemicals would be capable of inducing the same *number* of tumors whether it was spilled into a coffee urn or diluted a million-fold into a supply of drinking water. The difference lies in the size of the endangered population, and the relative risk to the individuals in the populations. Responses of this kind are frequently referred to as *linear;* however, they may not actually be linear except over a restricted range at low doses. The essential fact is that there is no dose so low that one can say that the effect is zero. It certainly will be the

case that there is a dose so low the induced effect is much less than the spontaneous one. In this case, one would not be able to observe the induced effect. However, one could infer its existence and could calculate the number of affected individuals.

The second kind of dose-effect relationship is characterized by S-shaped curves. Phenomena described by this sort of relationship — such as the action of poisons, some carcinogens, etc. — show an important concentration dependence, and there is a " threshold concentration " below which the agent is essentially without effect. S-shaped curves describe phenomena in which many molecules, or other particles, must act in concert to be effective. Simple dilution or dispersal of a substance decreases the probability that the number of particles required to do damage will be present in one place at the same time, thereby rendering the substances harmless (assuming there is no physical or biological mechanism for re-concentrating it).

The biological basis for the two different types of curves is not difficult to understand. Linear dose-effect relationships describe situations in which single packets of energy (photons, X-rays) or matter (molecules, viruses) are able to damage uniquely important molecules or cells in an organism. Such sensitive components include germ cell chromosomes, which contain the genetic information for the next generation, and stem cells in the embryo, which differentiate and multiply to give rise to significant portions of tissues or organs.

S-shaped, threshold-type dose-effect relationships indicate that a great number of molecules within a cell, or a great number of cells within an organism, must be attacked before damage can be seen at the organismal level. Metabolic poisons, such as arsenic or carbon monoxide, fall into this category because they must attack many molecules in many clees before they do any real damage.

It must be noted that for each different effect an agent has, there may be a different dose-response curve. For instance, while there may be a threshold concentration below which caffeine is not a stimulant (S-shaped curve), single caffeine molecules may cause mutations (linear relationship). Thus, when testing agents, it may be important to measure the entire spectrum of possible adverse effects.

The constant exposure of huge populations to small amounts of many chemicals makes it essential that we know whether such dosages affect small fractions of the population. The need for more sensitive tests has led to the use of insects, tissue culture cells, or micro-organisms in place of vertebrates, but it is often questionable whether results obtained from these experiments can be applied to man.

A convenient and often satisfactory alternative to testing enormous populations is to do experiments at dosages high enough to affect several per cent of a moderately-sized population. If one has reason to believe that the dose-effect curve is linear, the effects of low dosages can be estimated by simple extrapolating the empirical curve back to zero dosage. In this way, one can estimate the dosage that would adversely affect smaller fractions of the population than could ever be determined experimentally. If, on the other hand, the dose-response curve indicates a threshold, it is usually possible to determine the approximate " no-effect " level and then estimate the dosage to which humans can be safely exposed. There are, however, extremely complex theoretical and practical problems involved in the extrapolation from animal experiments to the effects on man. Since different species have different enzyme systems which can detoxify foreign poisons, there is no simple way of estimating the corresponding dose between animal and human effect. The usual basis of such extrapolations is to assume that there will be a corresponding effect when the same amount of the chemical is applied per unit weight of the organism. Although this method may be the best we can use at the monent, its many pitfalls must be kept in mind and efforts must be made to collect and correlate human effects whenever they occur.

A second way of sidestepping the difficulties of determing the low dosage-effect is to learn enough about the fundamental biology of the processes involved so that one can predict on theoretical grounds the way in which the substance acts. If we know the mechanism, it is often possible to predict whether a dose-effect relationship is linear or has a threshold. For instance, if it is discovered that a chemical directly modifies DNA, one would predict that the mutagenic effect of such a chemical would increase linearly with dose. Efforts could then be made to exclude that chemical from the environment. According to a recent report, sulfur dioxide, a common air pollutant, may be just such a chemical. Alternatively, a chemical may be thought to inhibit an enzyme. Because there may be thousands of these enzyme molecules in a cell, and millions of cells in an organ or organism, only large amounts of the chemical would have a significant effect on an organism (S-shaped curve with threshold). Such a chemical, if sufficiently dilute, could be tolerated in the environment. Hence, some organophosphates which are similar to nerve gases can be used safely as pesticides, because the field concentration is kept well below the levels which would be toxic to forms of life other than insects, and because the compounds are rapidly degraded by moisture.

Despite the conceptual simplicity of the experimental determination of a dose-effect relationship the practical problems are enormous and in no case is there sufficient data to allow a real decision to be made as to whether

a threshold exists for any chemical teratogen if it is applied at low dose. The answer may be different for different chemicals and for different species, but it should be possible with some chemical teratogens applied to small organisms to make the same type of determination which demonstrates a non-threshold effect of ionizing radiation in producing mutations by using bacteria as the test organism. In our laboratory we have started a series of experiments with a small tropical fish and with two small invertebrate species which should lead to a more sensitive test for chemical teratogens. Obviously, these organisms cannot be used as test organisms to screen chemicals since there is no reason to think that sensitivity or lack thereof for these organisms would correspond to sensitivity for man. On the other hand, it should be possible to improve our understanding of the basic mechanism by studying a dose-response curve in these small organisms.

One of the major interests of our laboratory is the extent to which genetics, nutritional and environmental factors can effect alternations in the structure and connectivity of a nerve net. The investigations must be made with organisms in which these factors can be controlled as fully as possible. In addition ,the methods used for obtaining and analyzing the three-dimensional structures must be as powerful as we can make them. I will try to indicate how these systems can be used in studying various problems in chemical.

The Organisms.

Three animals are currently being used in these studies. One is the rotifer, *Asplanchna Brightwelli*,an extremely small fresh water invertebrate with a total of 206 cells in its brain. A second is the water flea, *Daphnia magna*, which has several thousand cells in its brain, and has a compound eye with 22 lenses each surrounded by eight receptor cells. These 176 receptor cells send fibers to the brain with no interconnections between them before the fibers branch in a complex network within the optic tectum (that portion of the brain which is responsible for the first processing of optical information). Although the entire brain of Daphnia is too complex for analysis with our present methods, it is possible to analyze the initial branching and connections as the fibers from the eye interact with 110 cells which form the front part of the optic tectum. The third organism which we have recently added to our collection is a small tropical fish, *Poecilia formosa*. The total brain of this small vertebrate is many orders of magnitude more complex than either of the invertebrates; however, it has two large cells, the Mauthner cells, which have been extensively studied in other fishes and which can be located easily.

Since these cells receive inputs from many separate fibers, there is a highly complex but very specific structure composed of the Mauthner cell and its connections on each side of the fish. These two Mauthner cell complexes on opposite sides of the fish show at least approximate bilateral symmetry, and the extent of the symmetry can be studied in detail.

These three organisms span the animal kindgom, with the small organisms having only a few well-defined behavioral patterns and showing no evidence that they can learn anything even after extensive efforts at training. The fish have complicated behavioral patterns that are modifiable with both long- and short-term variations induced by training.

All three of the animals are able to reproduce parthenogenetically in such a way that no genetic material is contributed to the offspring by the males. The offsprings under these circumstances, must be female, and are genetically identical to their mothers. It is therefore possible with each of these organisms to obtain an unlimited number of individuals which although they are born at differen times, are genetically related as though they were identical twins. Thus in a population of genetically identical animals we can study the variations which arise by differences in environmental stimuli, nutrition or random chance variations during growth and development .

Data Collection - Image Combining.

The primary process involved in determining the three-dimensional (3-D) structural information of nervous tissue is the fixing, embedding and cutting of serial sections of the tissue which are then photographed with a light or an electron microscope. The dimensions are such that several hundred to several thousand photographs are necessary to record the structures of interest in a single animal. In order to handle the photographic information in an orderly way, we have developed a procedure for copying the photographs on to a motion picture film strip, with the picture of each section being aligned manually with the one in front of it (as described by Levinthal and Ware, Nature, 1972). The result of this process is a motion picture film strip which can be projected so that the observer has the illusion that he is " walking through the animal ". Three-dimensional relationships become apparent as the movie projector runs forward and back.

Data Collection - Computer Recording.

In order to record the 3-D relationships which an observer can " see " in the movie, the projected image is superimposed on the image of a computer-driven oscilloscope display. The obersver can draw in 3-D space by moving a device with two wheel-driven potentiometers which control the position of a spot on the oscilloscope. This indicates the x and y coordinate of the point to be recorded; the frame number of the movie determines the z coordinate. Since the movie projector is driven by the computer, the full 3-D information is available in digital form.

Features of the tracing and display programs include provisions for nerve file editing, for comparative displays of one side of an animal with a mirror image of the other, and for making visual comparisons of the same structure in different animals, with facilities for orienting and scaling one animal with respect to the other. These programs and several other features for matching recordings obtained from animals at different ages of embryological development will be described in detail elsewhere.

Results.

Substantive results are beginning to be obtained on the small invertebrates, and the methods for handling the fish are almost completely established. The questions being investigated at this stage are: (a) How nearly identical are the nerve nets in the brains of genetically identical animals when the animals are raised in essentially constant environment: (b) To what degree of fine structural detail does the bilateral symmetry of the organism extend? (c) Since precise bilateral symmetry cannot exist at the mid-plane of the organism, does the breakdown of symmetry show an organized pattern, or does it vary from one animal to another? (d) Is the developmental process in the organism completely orderly in the sense that it is the same in each animal, or are there random variations? (e) To what extent can one understand the mechanism by which the nerve net is formed by observing the anatomy of the partially formed structure? As would be expected, the degree of similarity, as well as the fine structure symmetry is much higher for the smallest animal studies, that is the rotifer (Ware Ph.D. Thesis, MIT, 1972), than it is for the somewhat more complex water flea.

We have not yet started to use these experimental systems for studying chemical teratology. However, to the extent that low level experiments will

be necessary in order to determine dose-effect proves it seems reasonable that systems which have a low natural background variation will be essential. The experiments we are now doing are necessary first steps before systems of this type on organisms with no genetic variation can be used for experiments in which induced variations are studied. The tropical fish seems to be the more likely to be useful for teratogens studies than the invertebrates. However for future work it would obviously be extremely helpful if one could find an organism closer to man which has the advantages of genetic similarity in a large population and ease of handling in the laboratory. At the moment it does not seem likely that we will find an organism which has all of the desirable properties. Therefore, experimental teratology will continue to use whatever compromises are available.

DISCUSSIONS

V. INGRAM : Can animals, such as small vertebrates, with small endogenous background of abnormality be used for screening for teratogenicity ? This morning we heard that spontaneous birth defects in man is at least an order of magnitude higher than in animals. Therefore animal systems may be not useful for such screening.

C. LEVINTHAL : I don't think I said and I certainly did not mean to imply that one can use these small animals as the only test system for screening chemical compounds. That is clearly out of the question since species differences are must too gross to allow the use of small invertebrates for this purpose. I think that the small organisms can, however, be used in trying to obtain answers to some of the basic biological questions which would help in making extrapolations from high to low doses. In trying to design a screening system for chemical compounds in the environment one will have to use every technique which is available including extensive tests with small organisms and cells in tissue culture. It is obviously not possible to carry out statistical meaningful tests with the usual laboratory animals on more than a very small fraction of the total number of chemical compounds to which human populations are exposed. With regard to the question of whether congenital anomalies in humans are much higher than in animals, I think others here could answer that better than I, but I think it is possible that the difference is that one has been able to detect chromosomal abnormalities in early abortions of humans much more effectively than in any animal experiments I know of.

J. WARKANY : I enjoyed your movies very much and I want to point out that a few things that you mentioned have been done in the last 30 years. I am not sure if you are familiar with the entire literature. However, what I want to ask you now... (Which ones ?)... experiments with drugs, the drug gets through to the embryo through the placenta or not, you just said...

C. LEVINTHAL : As far as I know, there has been no experiment done which would give an indication of what the shape of the dose response curve is for any chemical teratogen. Am I wrong in that ?

J. WARKANY : I think you are.

C. LEVINTHAL : Which ones, and what is the answer.

J. WARKANY : Thalidomide - there have been response curves with all sorts of teratogens.

C. LEVINTHAL : And what is the answer ? Is there a threshold or not ? But I'm sorry, this is an important point. In order to answer the question of whether or not there is a threshold one has to know whether one is failing to see an effect at low dose because you are getting into the noise of the spontaneous backgroung, or whether you are not seeing an effect because there is really a threshold. I would like to point out that in answering this question with regard to radiation it took very large experiments and considerable subtlety in experimental design. To the best of my knowledge neither of these has been used in experiments with thalidomide or any other chemical teratogen.

J. WARKANY : What is the proof of Dr. Levinthal's statement at the Press Conference that herbicides have increased the incidence of congenital malformations in Vietnam ?

C. LEVINTHAL : They're certainly not my experiments. I am sure you know the data as well as I. The fact that dioxin is a powerful teratogen and that dioxin is a rather common contaminant of the 2-4-5-T and other herbicides that were used in Vietnam, I think is quite clearly established.

The question of whether there has been experimental evidence on people, you know as well as I, is a debateable point. The fact that compounds which are now prohibited for use in the United States because of their suspected teratogenic effect were used in very large quantities in Vietnam as I assume you also know. So I don't know what we're arguing about.

J. WARKANY : Do you have evidence that herbicides have increased the incidence of congenital malformations in Vietnam ?

C. LEVINTHAL : I just told you what the evidence is. I think the statement I made, was that agents which were teratogenic, were used in Vietnam in large quantities. I believe this is a correct statement.

S. LARSSON : The statement made by Dr. Levinthal on the 245-T as teratogens are not relevant in view of the pertinent literature and the concept made by expert groups in Sweden or in the U.S.A. The content of dioxin has been considered.

C. LEVINTHAL : The only time I mentioned 2-4-5-T was just now when the question was asked, and I specifically said that to the best of my knowledge the data one was concerned about was the data concerning dioxin which certainly was a contaminant of the material which was used in Vietnam over quite a long period. I did not say that 2-4-5-T itself was a teratogen, but that dioxin is a teratogen and to the best of my knowledge, there was dioxin in the 2-4-5-T that was used.

S. LARSSON : May I ask, or comment upon again, in this review also dioxin as a contaminant of 2-4-5-T has been considered so this is included and the Committee have thought about it. There is nothing new in your statement.

C. LEVINTHAL : I'm sorry, are you saying there is no dioxin in 2-4-5-T ?

S. LARSSON : There is dioxin and we are aware that it can cause teratogenic defects itself. Also in combination with 2-4-5-T, but these facts have been considered by the different committees, by the different experts, so what you are saying is nothing new.

W. MCBRIDE : Defoliants have been used extensively in Australia to kill lantana in extensive grazing areas of Australia, this was sprayed by helicopter. Domestic animals have an incidence of congenital anomalies as do humans. In the areas sprayed there was not an increase in the incidence of congenital abnormalities among the cattle or the humans living in the area.

C. LEVINTHAL : Do you know the concentration of dioxin in the 2-4-5-T that was used ? How much of the material was ingested by the pregnant women and how many patients were studied ? With what control group were the exposed patients compared ? There is not good positive evidence of the teratogenic effect on people in Vietnam and I don't think that the negative evidence in the exposed Australian population is very good either. Therefore, it seems to me that we are left with the studies on experimental animals with which to make a judgement as to whether or not these materials should be used.

L. WOLPERT : I don't understand the relevance of the daphnia studies to this meeting. It seems a random choice from a very large number of different systems and it seems a bad one since it is possible that the development of its nervous system involves a precision not found in mammals.

C. LEVINTHAL : As I pointed out before in answering Ingram, no one, certainly not I, suggested that one would use small invertebrates as the sole means of screening. What I am saying is that if one is trying to find out

whether environmental alterations, whether they be chemical or physical alterations, can, in fact, produce subtle changes in an organism. But one will have to, use organisms which have a small genetic variation to start with. Now, clearly, I agree that if one is trying to get at this question directly, one would much prefer to use a vertebrate, which is one reason why we have started to work on P. Formosa the tropical fish. For this kind of a question one would clearly want to use either one of the fish in which one can get parthenogenesis or one of the other large organisms in which one can get parthenogenesis, because under these circumstances, I think one can look for subtle changes and ask whether or not it is possible to induce them at what stages it is possible to induce them.

L. WOLPERT : I suppose what I am really asking about is whether it is really meaningful at this particular stage to look for these very subtle changes. I am really not at all persuaded that this is really an important question at this stage.

J. WARKANY : I do not attack Dr. Levinthal, but those neoteratologists who do not know anything about congenital malformations but make statements that are political rather than biological.

K.H. DEGENHARDT : I can substitutes your statement concerning a linear dose-response connection between X-ionisation and congenital malformations in the skeleton of the inbred strains C57BL in mice. There is a clear threshold phenomenon in inbred strains of mice. Concerning the dose-response question between 5 fluoro-deoxycytidine and for example malformations of the vertebral column. I may ask you, what is the main point of relevance for the human being, using daphnia as a testing system for teratogenicity ?

M. WINICK : As a clinician, can I rise in semi-defence of Dr. Levinthal, because there are some things I disagree with, or I have questions about, but first of all, to raise at this point the relevance to human malformations really isn't the issue : the issue here really has to do with markers and I think the difference between what Dr. Warkany was referring to and what some other people are doing at the present time in terms of teratology, I don't mean the group necessarily that Dr. Warkany was referring to, is simply a difference in the markers they are looking at. There is no difference in many experiments in terms of the experiments. In one case one is looking at morphologic markers, now people are looking at biochemical markers, but it is a question of the marekrs. Now what you are suggesting is, I assume that we can shorten some of the markers that we might be able to develop by looking at a system which is a simple system to look at and which has less of a background : I think that's a reasonable, a good suggestion but I would

like for you to get a little bit more specific in terms of the markers you are talking about, subtle changes don't really mean anything to me. What I want to know is, what kind of markers should we be looking for in these systems, and in terms of a marker of development in daphnia which we may be able to adapt to other systems. But in terms of relevance to humans I think we are raising a point here which — you know, we can't decide what's relevant and what's not relevant.

C. LEVINTHAL : Let me try and answer these two comments together. I think on the question of relevance, the issue that seemed to me to be important, is the issue which we started to discuss after Winick's paper of yesterday. To what extent, in animal systems, can one detect changes which lead to different learning abilities in animals which have been subjected to malnutrition during certain periods of development. If one is going to try to answer the question of whether or not malnutrition during certain periods of development can, lead to a significant reduction in learning ability, it seems to me extremely difficult to carry this out as epidemiological experiments with people, and not, in principle, difficult to carry out on animals if you start out with animals that have a reasonably well defined genetic background. I think that this kind of experiment with inbred mice, with parthenogenic fish, with any number of other organisms, are a class of experiments which are important for social as well as for scientific reasons. I don't think we can even begin to answer the question of whether or not environmental effects like malnutrition or environmental insults can produce effects of this kind, if we restrict ourselves to studying the problems in humans.

L. WOLPERT : I can't again understand your insistence on constant genetic background. There are well-defined techniques for picking up differences against a variable background. More seriously many inbred strains show greater morphological variability than the wild type population.

C. LEVINTHAL : This is not correct. If you're talking about organisms which reproduce parthenogenically their similarity is that of identical twins.

S. LARSSON : No relation to the practical problems concerning the dose levels when you have to give advice about the risk for malformation. Moreover, the methods suggested for placental transfer, microsomal enzyme induction of drugs are already used in teratology. So I do not find any new and realistic in his advice to the progress of teratology and drug testing.

S. BENNETT : I wish to encourage Professor Levinthal to continue his studies of the factors affecting development in Daphnia and Rotifera. Perhaps we

physicians need to be reminded that the relevance to medicine of the work on genetics of neurospora by Beadle and Tatum was not widely appreciated by academic physicians at the time it was under way. One can expect that deeper understanding of control mechanisms in developmental biology will emerge from studies with organisms such as Dr. Levinthal has shown us. They develop in a rather uniform matter, and large samples free of genetic variation can be obtained. I shall await with interest the results of these studies and be patient if application of the results to problems of human teratology do not emerge immediately.

M. WINICK : Enzyme systems for handling of drugs may be effected by such nonspecific changes as diet during infancy. It has been recently shown that malnutrition during this period that I was talking about, will cause changes in the enzyme systems, the microsomal enzyme systems which metabolize drugs, and that these microsomal enzymes changes are premanent even after you rehabilitate the animal. So that it is possible to induce enzyme changes of a permanent nature by things as non-specific as protein caloric malnutrition of the animal. So that you really have to know when you're looking at an environmental teratogen whether or not this is working specifically or whether or not this is creating some kind of situation in the animal which is indirectly then working on an enzyme system. A sick animal, from any cause, may very well have a different kind of enzyme induction than a well animal.

K. HIRSCHHORN : With reference to the example by Dr. Bennett of Beadle and Tatum's work, it should be remembered that an academic clinician named Garrod first invented the one gene-one enzyme hypothesis in 1903 and that human geneticists recognised the relationship of the work on Neurospora to inborn errors of metabolism as it was being done.

L. WOLPERT : It's just on a question of fact. Do I understand Dr. Levinthal to say that the connectivity of invertebrate nervous systems — or any invertebrate nervous system is always the same ?

C. LEVINTHAL : What I said was that the connectivity in the rotifer, as far as we have been able to study it, is the same from animal to animal.

I have one last comment which will be very brief. I was going to mention an example different from the one Dr. Bennett used which I think is even more relevant. That is, the discussion which took place between Dr. H.J. Muller and most radiologists over many years. In that confrontation I think the fury of the radiologists at being told that they might have been killing more