people than they were helping was not to be matched by that of the teratologists at this or similar meetings.

Dr. MOSCONA : It might be useful to give some thought at this meeting to the need for suitable test systems for screening for potential teratogens. These systems should be based on the essential processes of development which, if disturbed, may lead to malformations. Such screening " model " systems would be a first step towards testing on original embryos and towards analysis of their relevance as teratogens in human development. The major developmental processes which should serve in screening of potential teratogens are : (1) Chromosomal structure and number, (2) cell replication and growth, (3) morphogenetic cell measurements, (4) synthesis of specific macromolecule (cell differentiation), (5) specific cell interactions. There are numerous experimental mammalian and other systems in each of these categories from which one could select those most suitable as " models " for primary screening of potential teratogens. Some of these systems lend themselves to quite sophisticated cellular and biochemical analysis. It seems to me that a combination of such an approach with clinical studies, plus continued exploration of such " unconventional " systems as discussed by Levinthal will have to be an essential part of the overall strategy for the surveillance of the environment for teratogens and for the understanding of the detailed mechanisms of teratogenesis.

W. MCBRIDE : You have mentioned ionizing irradiation on several occasions. What would you consider a safe dose level of irradiation when used for diagnostic purposes during human pregnancy.

C. LEVINTHAL : I don't think we know the answer to that question. I think it is an important question, but from any experiments I have seen, I don't think we have any basis for saying it is above zero, or what the level is.

W. MCBRIDE : But surely you must have some idea. Would you be willing to X-ray a woman during pregnancy ? I mean, this is the question that a woman is going to ask you, and you can't just say, " we don't know ", at this stage and we're thinking about it.

C. LEVINTHAL : I'm sorry but I try to work as a scientist and as a university teacher and if I don't know the answer to a question, even a very important one I can say that I don't know.

J. MILLER : It seems to me I've heard all of this before. At the 2nd International Congress of Congenital Malformations held in New York in 1963

Clarke Fraser of McGill reviewed a variety of test systems for teratogens and discussed a number of factors which could influence teratogenic activity. He concluded that the only reliable test system was the intact human being. Professor Barnes of Johns Hopkins has seconded this opinion and I believe many teratologists would agree.

Although non-human test systems (cell systems ,other animals, etc.) will provide much useful information in fundamental developmental biology, I think we must face the dramatic implication of Fraser's conclusion. In all of the discussion here we have not even touched upon this subject, let alone faced it realistically and at the rate we are proceeding we shall never do so !

C. LEVINTHAL : Can we stop this discussion now because I think this particular note is one where it is appropriate to introduce Dr. Monroy, who will be talking about a phenomenon which I think even the most optimistic epidemiologist would agree would be difficult to unravel by an epidemiological approach.

BIRTH DEFECTS, DEVELOPMENTAL CONTROL, AND THE SCREENING OF TERATOGENS

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There are many uncertainties in standard methods of screening teratogens by their administration to pregnant female mammals. These include size timing of dose and ignorance of either the target tissues or response to the teratogen. It is extremely expensive and inefficient to eliminate these uncertainties by systematic use of conventional procedures. Therefore we propose a simpler and more direct method which would also give information about normal development. The method depends on a logical analysis of the control of development which originated in the work of early embryologists.

Birth Defects and Developmental Control.

All multicellular animal development proceeds by numerous repetitions of only 6 basic cellular events : nuclear or cell division, growth, differentiation, movement, contact formation or breaking, and secretion. These are unitary, simple, and universal at the cellular level or above. Even the details of their intracellular mechanisms are quite similar in different embryos.

This conference has focussed almost entirely on the unreliability of development, emphasizing such astonishing statistics as the survival of only development is reliable. Embryos with identical genomes develop into nearly identical adults. The most impressive example is the physical and emotional similarity of human identical twins reared apart. The occurrence of many repetitions of the unitary events which are required for development must be subject to control in time and in space for such reliability to be possible.

Control is exerted at all levels of organization. There can be intracellular control in which each cell in a field develops independently according to an internal program derived ultimately from the genome. The can be field-wide control in which cells functionally coupled to their neighbors interact and

influence one another's development, as in morphogenesis and spatially patterned differentiation, or there can be control of a process throughout the organism as in the initiation of metamorphosis by ecdysone. Control at the higher levels of biological organization proceeds ultimately by molecular mechanisms and must involve molecular transport between cells. The elucidation of developmental control systems and mechanisms is central to embryology and requires research properly balanced between the molecular and biochemical, for identification of agents, and the experimental embryological, for the identification of modes of intra and multicellular action.

There is more or less universal faith in the existence of developmental control at the molecular-genetic, intracellular level, and some information about mechanisms is emerging. At the higher levels few intercellular control agents have been identified. These include auxins in plants, ecdysone in insects, and cyclic AMP in the cellular slide molds. How auxin generation and transport gives rise to pattern formation in plants is just beginning to be understood. Ecdysone acts as a nonspecific switch. It is only for the cellular slime molds that some quantitative experimental and theoretical analysis of a multicellular developmental control system has emerged. Nevertheless a substantial amount of indirect experimental evidence exists for multicellular developmental control systems the logical analysis of which is quite convincing.

How can we reconcile the evidence for control systems leading to reliable development with the evidence for a high proportion of early embryos being defective ? We suggest a reconciliation along the following lines. First, the developmental control systems apparently can exert control only within a well-defined range of variation of the biological variables to be controlled. Regulation, that is the development of nearly size-independent patterns or morphologies, illustrates this; a very good example is provided by Horstädius' studies of the sea urchin. Second, some events at the beginning of development are at most weakly controlled. For example, the first cleavage plane in the newt, Triton taeniatus, always contains the animal-vegetal axis. In contrast, it can have any orientation with respect to the dorso-ventral axis. Normally it contains the dorso-ventral axis, but there is a finite probability that it will form perpendicular to that axis. If blastomeres resulting from cleavage with normal or near normal orientation are separated regulation occurs and two half-sized but otherwise normal embryos result. On the other hand, if two blastomeres resulting from cleavage perpendicular to the dorso-ventral axis are separated, the dorsal blastomere develops into a normal but halfsized embryo whereas the ventral one is blocked at the gastrula stage and does not neurulate.

We suggest (1) that such variability is common at the earliest stages of

development, and (2) that therefore there is some probability that conditions of the embryo will be outside the range of regulation of the control processus governing later stages of development. In this way one can reconcile a high defect rate early in embryogenesis with the later precise operation of developmental control processes within normal limits. Moreover, such a high defect rate need not have a genetic origin.

Screening of Teratogens.

In the above view the high early defect rate is intrinsic to development. It is unlikely to be improved by treatment or caused by teratogens or maternal condition. Moreover, it is also possible to reach conditions of the early embryo which lie marginally within the range of operation of the control processes, leading to embryos that survive to term with inborn errors not attributable to defective genes, specific teratogens, or maternal conditions. Such errors would contribute an irreducible minimum below which we could not reduce the incidence of birth defects by active steeps. What is left for attack are genetic defects both in relation to function and to development, and teratogens acting subsequent to those stages of development leading to the intrinsic variability we have proposed. We are concerned with the latter.

In our view a teratogen can interfere with either the occurrence of the unitary events or with the superposed control systems. Indeed, such an agent would be very useful in elucidating the nature of the intercellular control systems. Thus as teratogen screening program which both reduced the uncertainties alluded to in our introductory remarks and established the mode of action of the teratogen would have both immediate social value and long-ragne scientific value. We think this can be achieved by taking advantage of the universality of the basic developmental events. Thus, we should select a convenient organism in one particular developmental state at which one of the unitary processes is clearly isolated and well displayed, apply the suspected teratogen locally in an appropriate range of doses and observe the consequences, i.e., the dose-response relationships at all subsequent stages of development.

The paradigm of such an approach could be the beautiful and simple experiment on the chip limb in which Wolff demonstrated the mode of action of thalidomide. The apical crest of ectoderm on the limb bud acts as a classical organizer and is a seat of control of the development of the limb. The mesoderm immediately under it proliferates in response to the apical crest, this proliferation being the unitary event in our view. Wolff excised the apical crest, exposed it to thalidomide, and returned it. Normal development ensued. He then excised the underlying mesoderm, exposed and returned it.

Limb development was prevented. Thus the mode of action of thalidomide was established as inhibiting proliferation of the limb-bud mesoderm.

We suggest that a suitably designed battery of experiments of this general class would simultaneously screen teratogens efficiently and yield information on the mechanism of action of the teratogens thus discovered. One would incorporate in such a battery experiments aimed at a representative sample of the developmental events containing all of the unitary processes in varied circumstances as well as the various categories of control system as far as these are understood. It is the fundamental developmental *processes* that should be exposed to teratogens, rather than whole embryos whose complexity may obscure or shield an effect. It is possible that an agent which interfered with a developmental process in one embryo would not affect it at all in another, perhaps because its target is protected by a permeability barrier. Implicated agents could then be tested by more conventional means which mimic more closely the conditions of human exposure. One possible way to minimize invalidation of this screening procedure through differences between human biochemistry and that of the experimental animals is to include tissue cultures of human cells among the test systems.

The work on which these remarks is based has benefitted from partial support by the National Science, Sloan and Ortho S. Sprague Foundations.

A MULTIREGIONAL PROSPECTIVE INVESTIGATION (*) ON PREGNANCY COURSE AND CHILD DEVELOPMENT; A FIRST PRELIMINARY EVALUATION

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Abstract.

This evaluation is based on a collection of about 6000 pregnancies. For statistical analysis we defined special " influencing factors " and special " goal variables ", which have been examined for correlations. Main attention was focussed on the first trimester of pregnancy especially regarding drug intake, viral infections, uterine bleeding and the EPH syndrome. The goal variables refer to the pregnancy course and the outcome of pregnancy, including child development up to the age of about 3 months. About 4000 pregnancies have been evaluated with regard to 65 histomorphological placenta variables. 3000 pregnancies were analyzed with regard to distributions of titres in the antigen-antibody reactions against rubella-, mumps- and cytomegalovirus in the first trimester and conversions or titre changes in the following trimesters resp. after birth. The same has been done for toxoplasmosis. Drugs were classified into 9 broad and 50 more differentiated groups; 5 single drugs were selected for special evaluation including time of intake and basic pathological situations.

The preliminary results give some hints in order to estimate environmental as well as specific genetic influences on the outcome of pregnancy.

The final aim of the science of teratology should be to prevent the origin of congenital malformations. Great efforts are made in many countries to elucidate the very complex mechanisms of prenatal development in human beings, as they are directed by the genome and manifold influencing factors in the environment. It is well known, that the early period of organo-genesis is the most vulnerable one. In addition there is strong evidence for the

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assumption, that a big part of congenital malformations, especially those which have social medical importance, might be caused by a complex interactions of genes and exogenous influencing factors. Prospective investigations are of high value to collect reliable data about the time around conception and the following weeks of early pregnancy. For this purpose a committee of the *German Research Foundation* initiated a prospective longitudinal investigation in the year 1963. The study is being performed by the collaboration of 18 clinics of obstetrics and gynecology and 18 clinics of pediatrics and 30 special laboratories. Gravidas are registered as early in pregnancy as possible, at least before the 12th week after the last menstrual period. During pregnancy physical reexaminations are performed every 4 weeks and in addition the gravidas fill in a diary. Newborns are examined twice, and the further development up to 3 years of age is controlled by experienced pediatricians. The whole body of informations is collected in prepared documentation sheets. Storing of data and statistical analysis are in hands of Prof. Koller, director of the institute for medical documentation and statistics, Mainz University. Up to June 1th 1971 there were 12 652 gravidas registered, more than 1 000 of them twice or more times. 9 292 live born and 102 stillborn newborns and 1 089 abortions were documented. For preparations and performance of the preliminary evaluation 11 groups of experts were named with regard to special groups of variables, e.g. drugs, toxemia, viral infections, toxoplasmosis, placenta a.s.o. (table 1).

TABLE 1

Preliminary evaluations of the prospective study.

Influencing Factors	*Variables*
Drugs	Placental Morphology
Toxemia	Delivery
Bloodgroups	Prematurity, immaturity
Rh-Subgroups	of the newborn
Anemia	
Diabetes and other	Spontaneous abortions
Metabolic diseases	Congenital malformations
Virusinfections	Any physical deviations
Toxoplasmosis	

The study population may be selected in the sense of a higher frequency of gravidas with planned children (table 2). Two thirds of first and second-parae belonging to the age group of 20 to 24 years and three quarters concerning the age group of 25-29 years expressed their wish to have a child. The age distribution of the gravidas was approximated to that of the general statistics of the German Federal Republic in 1967 (table 3). The frequency of

TABLE 2

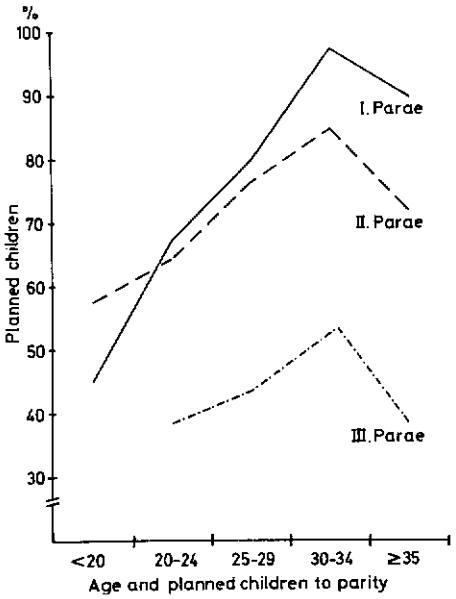

TABLE 3

Age pattern of pregnant women in the prospective study compared with the general statistic of German Fed. Republic.
No. of pregnant women : 5.486

Age groups in years	DFG-Study %	Gen.-Statistic %
< 20	5.7	6.5
20-24	27.2	26.7
25-29	39.9	35.1
30-34	19.5	20.3
35-39	6.2	8.5
> 40	1.5	2.9

primiparae seemed to be higher than in the general statistics. Approximately equal were the rates of stillbirths and infants, who died within the first 7 days after birth. Regarding the causes, why the gravidas visited the clinics of obstetrics and gynecology it could be found, that selection of the registered study population seemed not to be as high as originally supposed (table 4). At least 75.6 % of the women asked for diagnosis of pregnancy. Because of statistical evaluations it is important to know, how often events may be observed which could possibly influence pregnancy and fetal development (table 5).

TABLE 4

Motive of registration within the first trimester of pregnancy.
No. of pregnant women : 5.347

Threatened abortion	3.8 %
Treatment because of sterility	2.8 %
Treatment because of other motives	9.8 %
Troubles because of pregnancy	7.9 %
For diagnosis of pregnancy	75.6 %

TABLE 5

Incidences of special events in pregnancy.
No. of pregnant women : 5.486

Diseases with fever during the first 4 weeks	10.2 %
Other diseases during the first 4 weeks	8.4 %
Diabetes	1.0 %
Thyroid diseases	12.6 %
Heart- and circulatory diseases	6.6 %
X-irradiation up to the 20th week	10.7 %
Any drug intake during the first trimester	79.7 %
Bleeding	23.2 %

This year the first preliminary evaluation has been performed on the basis of approximately 600 tables, with two dimensional associations of specific variables; in special cases multidimensional associations were tested. We agree with Yerushalmy's statements concerning the statistical evaluation of the prospective study on pregnancy and child development in Northern California, that we should use utmost criticism in the interpretation of associations with statistical relevance. As a basic rule we have to search for manifold sources of selections in self-formed subgroups as formed in the study population. Groups which are being compared in one characteristic trait may not be alike in all pertinent characteristics. With these well-known difficulties in mind, we may present some observations.

The high frequency of drug usage during the first trimester of pregnancy was rather surprising. Analgesics/sedatives/hypnotics were ingested most (table 6). If we split the drug usage in 61 subgroups, the laxatives/purgatives are in the foreground. 80 % of all gravidas (ca. 5 500 cases) ingested drugs. A large number of variables were tested for associations with the nine drug groups und with 5 single drugs, which were selected because of the widespread use in our country. Only a few associations were found to be statistically significant. Further analysis, however, proved them to be at least questionable because of conditions in the background, which do not allow to interprete these associations as causative.

TABLE 6

Exposure to drugs during the first trimester of pregnancy. No. of pregnant women : 5.752

	n	%
1. Analgesics, Sedatives, Hypnotics	2.077	36.1
2. Vitamines, Anabolics, Minerals	1.426	24.8
3. Laxatives, Purgatives	1.108	19.3
4. Sex Hormones	1.006	17,5
5. Antiemetics, Antihistamines	844	14.7
6. Cardiac Glycosides Circulatory Drugs	685	11.3
7. Chemotherapeutics, Antibiotics	625	10.9
8. Corticosteroids	129	2.2
9. Other Hormones	89	1.5

No relevant association could be found between drugs and 9 specific types of congenital malformations. The incidence of the malformations in shown in table 7 in comparison with findings in the WHO-study of Stevenson 1966. The rate of all congenital malformations including minor defects was 6.4 % (4 880 cases).

TABLE 7

Incidences of certain types of congenital malformations in comparison with those stated in the WHO-study 1966

Types of malform.	DFG-Study (4.880 cases) %	WHO-Study (416.695 cases) %
Heart defects	0.246	0.074
Facial clefts	0.102	0.121
Dorsal clefts	0.307	0.259
Epi- and Hypospadias	0.143	0.061
Poly- and Syndactyly	0.369	0.127
Dysplasia and dislocation of hip	0.327	0.029

These negative results up to now should be regarded with great reserve and should not mean, that drug intake during the first trimester of pregnancy may not be of danger.

The search for virus infections concerned serological examinations in the gravidas 3 times during the course of pregnancy and at delivery and also from the umbilical cord blood of the newborn. It could be stated, that about 90 % of gravidas had antibodies against rubella. The conversion rate was 0.6 % (= 11 : 1964 gravidas). 2.4 % gravidas showed titer changes at least within

two classes. Concerning cytomegalovirus the rate of gravidas with antibodies was about 56 %, conversion rate 5.8 % (98 : 1687 gravidas) and titer changes 2.9 %. With regard to mumps virus in 48 % of gravidas antibodies were found, conversion rate was 12 % (= 181 : 1469) and titer changes 0.9 %. These investigations were performed under the heading of Prof. Haas Freiburg University and Dr. Enders in Stuttgart.

Among numerous variables so far tested with subgroups of gravidas with antibodies against the 3 virus infections mentioned above, only one significant positive association could be found concerning the increased frequency of stillbirths in gravidas with antibodies against cytomegolovirus in the first trimester of pregnancy. This has to be further analyzed.

With regard to toxoplasmosis 63 % gravidas had antibodies. One special antigen was used by all collaborating laboratories. No association could be found between high positive titers and frequency of abortions or stillbirths. But gravidas with low positive titers had a higher frequency of earlier stillbirth than those with high or negative titers. This should be assured by collecting more data.

The frequency of manifested diabetes was 1 % (= 44 : 4336). The wellwellknown risk for the outcome of pregnancy could be corroborated : the rate of stillbirth was 9.1 % in the subgroup of gravidas with diabetes against 1 % in the control group. There were positive associations between diabetes of the gravida and immature villi of placenta. A special investigation under the heading of Prof. Schöffling Frankfurt toge-ther with Dr. Staffeldt Berlin concerned 500 gravidas which were examined for unbalance in the carbohydrate metabolism by intravenous glucose application. In 350 gravidas insulin was measured by the way of immunological reactions. Thorough evaluation of the latter findings are in progress. The following could be stated :

1. Infants with heavy birthweight derived from gravidas with a pathological insulin secretion in a higher frequency than infants with normal birthweight.
2. Potential diabetic gravidas, e.g. gravidas with diabetic relatives of first and second grade, or preceding abortions and stillbirths or infants with heavy birthweight of more than 4 500 g, had a pathological glucose assimilation.

Further data are needed for tests of associations with statistical relevance especially concerning the health condition of newborn from gravidas with unbalance in the carbohydrate metabolism.

These are some preliminary results, which should be acknowledged cautiously. They may be valuable in comparison with results which were obtained in quite analogous prospective investigations of other countries.

OOGENESIS AND MALFORMATIONS

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Before starting my presentation, I want to make clear that I have never worked with mammals. The animals on which my colleagues and I have been working are sea-urchins, toads and lizards. Anyway, there are good reasons to believe that at least some of the data obtained can be applied to mammals.

Fertilization is usually considered as the starting point of embryonic development. This is true, in the sense that fertilization is the process that activates the egg. However, it is now generally accepted that the events of fertilization and early development (and I will tell a little later what I mean by « early development ») can be understood only in the light of what preceeds fertilization. That means that the events of fertilization and early development depend on and are conditioned by the past history of the egg; i.e. by the events of oogenesis. This is why oogenesis is now acquiring a key position in the study of embryonic development.

We may consider the following scheme. Oogenesis is followed by fertilization, and fertilization is followed by cleavage. What I propose now to show it that some events which take place during oogenesis determine and influence fertilization, cleavage and later development. And let us start by defining what we mean by oogenesis. Oogenesis in the process which leads from the primordial germ cell, still endowed with the ability of duplicating its DNA and of dividing and which we call the *oogonium* to a cell which while having lost the ability to duplicate its DNA, becomes very active in RNA synthesis and which we call an oocyte. Through the process of maturation, the oocyte acquires the ability to be fertilized and is now called ootide or egg.

Now, let me begin first by summarizing some of the basic observations which have established the role of the events of oogenesis in controlling early developments.

The best evidence comes from genetic studies. The first evidence to this effect came from some very beautiful studies of Boycott and his colleagues in Britain and of Sturtevant in the United States, which showed that the

direction of the twist of the shell (either clockwise or anti-clockwise) in the snail, Limnea, is determined by genes which are active only oogenesis. And no matter which the genotype of the father is, the direction of the twist of the offsprings will be the one which had been negetically determined during oogenesis. Hence, it is a strictly maternally determined character. Garen at Yale has now a number of Drosophila mutants which beautifully show how genes which are active only during oogenesis control specific stages of post-fertilization development.

Further evidence on the role of oogenesis comes from the study of hybrids. If you make a cross between two different species or between two different genera of echinoderms or amphibians you may obtain either a viable or a lethal hybrid. If the hybrid is viable, the paternal characters will only begin to show up fairly late in development, usually not before the gastrula stage. In the case of lethal hybrids, the hybrid may develop normally up to the blastula stage, and then will then die. These data already suggest that the egg is endowed with genetic information which is sufficient to support development until gastrulation. This is indeed the most critical stage of early embryonic development. The experiments which opened the way to the molecular approach to the study of early development and oogenesis, where some experiments in which actinomycin D was used. As you know, actinomycin is an antibiotic that abolishes RNA synthesis by interfering with the operation of the DNA-dependent RNA polymerase. The experiments with actinomycin, which were performed by Gross and Cousineau in 1963 showed quite clearly that sea urchin eggs treated with actinomycin since the time of fertilization (and it was certain that the actinomycin had altered the egg, because the RNA synthesis was almost completely supressed) develop to the blastula stage, in an essentially normal way. Again this suggests that at the time of fertilization, the egg has enough « information » to support development through the Blastula stage. However in order for the embryo to reach that critical stage that is gastrulation, new genetic « information » is required. In molecular terms this observation tells us that development to a certain stage is largely (indeed, not exclusively) supported by the RNA messengers which had been synthesized during oogenesis. Thus this implies that the egg must have a store of messenger RNA *which is not going to be used during the process of oogenesis but is meant for use after fertilization.*

As I told you before, in the transition from the oogonium to the oocyte the germ cell stops replicating its DNA. That means that while the oogonium is a dividing cell, the oocyte is a non-dividing cell. However, in the oocyte in which there is no overall replication of DNA, a limited area of the genome is actively replicated; this is the section of the genome which codes for the ribosomal RNA. This very interesting phenomenon has been studied in detail

first by Brown and Dawid in 1968 in the amphibians. Thus against the background of non-replicating DNA there is one section of the genome which actively replicates and thus becomes, as we say « amplified ». A very large number of copie of this section of the genome are thus produced, which are thrown into the oocyte nucleoplasm in the form of tiny bodies. The presence of these *multiple nucleoli* had been known for a number of years, but their significance was only clearified by the work of Brown and Dawid. Furthermore while the replication of DNA is mediated by the enzyme complex which is known as the systems of the DNA- dependent DNA polymerase, my colleagues Crippa and Tocchini-Valentini have recently (1971) obtained evidence that in the case of the amplification of the ribosomal RNA cistrons, the replication of DNA is mediated by a special type of polymerase which is RNA dependent. I can't go into the details now.

Which is the significance of the amplification ? The fact is that the oocyte has to provide itself with all the ribosomes which the egg will need to develop to the gastrula stage.

Thus not only does the egg stock-pile messenger RNA in sufficient amounts to support the development to the gastrula stage, but the development to the gastrula stage is also supported *exclusively* by the maternal ribosomes. All the ribosomes which are required to support protein synthesis to the gastrula stage are synthesized in the oocyte during a brief period of time. This is an additional example of the importance of oogenesis for early development.

Thus both the messenger RNA (mRNA) and the ribosomes that support protein synthesis (and hence development) to the gastrula stage are synthesized during oogenesis. However, for development to proceed further new mRNA and new ribosomes are required. The requirement for new mRNA is shown by the previously mentioned results of the experiments with Actinomycin which prove that if RNA synthesis is suppressed development stops before gastrulation. The best evidence concerning the need for new ribosomes comes from the study of an anucleolate mutant of the toal *Xenopus*. The work of Brown and Gurdon (1965) shows that the homozygous embryo develop normally until just past the gastrula stage. At this point, when the new ribosomes are needed, they cannot be provided because the mutant lacks the segment of the genome which codes for the ribosomal RNA (the so-called nucleolar organiser) and the embryo dies. At this point it may be pertinent to mention that the work of Woodland and Graham has shown that in the mammalian egg the store of maternal ribosomes is much smaller than in the other eggs studied thus far; indeed it has been found that the synthesis of the new ribosomes starts as early as the 4-8 cell stage. This observation has to be well kept in mind when extrapolating to the mammals results obtained

with non-mammalian vertebrates. It is of course also an interesting question whether the shorter dependence on the maternal ribosomes may be connected with the establishment of viviparity.

Let me quote two additional data which emphasize once more the importance of oogenesis in supporting early development. Smith and Ecker (1970) have evidence that the oocyte nucleus contains a factor which is important for normal cleavage to occur. Now this factor is released into the cytoplasm at the time of maturation. As you know maturation is a hormonally controlled process; one of the first things you see at the onset to maturation is the breakdown of the nuclear membrane which results in the admixture of the nucleoplasm with the cytoplasm.

The work of Smith and Ecker (1970) has proved that the cytoplasmic changes that are typical of maturation may proceed in the absence of the oocyte nucleus. And yet short of the admixture of the nuclear content with the cytoplasm the fertilized egg fails to cleave normally. Evidently, at the time of the breakdown of the oocyte nuclear membrane, some factor is released in the cytoplasm which is essential for normal cleavage to occur.

The second observation is due to Briggs who has recently discovered another mutant, also in an amphibian, which has allowed them to reach the important conclusion that the germinal vesicle contains a factor which is important for the egg to develop beyond gastrulation. The elegant experiments of Briggs and Cassens (1967) have shown that the injection in the mutant eggs of the content of the nucleus of normal oocytes allows these eggs, which otherwise would have stopped at gastrulation, to proceed in their development. I have quoted these findings to add further strength to the assertion that the events that take place during oogenesis control the events of early development. As you see, this is a wide open field.

There is one last question that may be important briefly to discuss in this context. I have started my story from the oogonium. The oogonium is, however, an already well differentiated cell which is committed to development in a certain direction; can we assume that the events of oogenesis may have an influence on the progenitor germ cells of the future generation? And the answer is yes. The best evidence available is derived from the insects.

In insects, following the third cleavage of the fertilized egg, i.e. at the stage with eight nuclei, one of them migrates to the hind pole of the egg, and becomes the progenitor of the germ cells of the animal. There is good evidence that the hind pole of the egg contains a RNA-rich material on the interaction with which the differentiation of the germ cell line depends. These observations show that the segregation of the germ cells occurs very precociously and the germ cells of an individual may thus be influenced in their

differentiation by the conditions of the cytoplasm of the egg from which they originate.

Now what about the Vertebrates and the mammals in particular? It is a common feature to all Vertebrates that their germ cells originate from an extra-embryonic territory. In the mammalian embryo they can be recognized for the first time in the wall of the yolk sac; than they slowly migrate and reach the somatic anlage of the gonad. Their earlier history is, however, not known. Some observations on the egg of Rana are worthy being mentioned. There is one line of evidence which I think is pretty good again in Rana, in the amphibian; and the evidence is that in Rana, there is again at the hind pole (the so-called vegetale pole) of the egg some nucleic acid containing material. Since we find it in the egg it must have been synthesized in the oocyte. There is evidence that this material is responsible for the segregation and differentiation of the germ line. We thus reach the important notion that the events of oogenesis, may influence the future development of the germ cells that will develop from that oocyte when it will become an egg and will be fertilized.

I want to repeat once again that thus far the experiments on the mammalian egg are rather difficult. Only recently it has become possible to obtain *in vitro* maturation and fertilization of mammalian eggs and this opens up new possibilities. Of course, the great limitation of the mammalian egg from the point of view of the approach at the molecular biological level, is the small amount of material that can be obtained. Refinement of the analytical techniques will hopefully allow to overcome this difficulty.

DISCUSSIONS

K. HIRSCHHORN : There is something, it is simply a question of fact, that has puzzled me for a long time in terms of the anucleolate toad. If, in fact, the store of ribosomal RNA is so important for early development and the genome is lacking for the production of ribosomal RNA later on, what makes the ribosomal RNA later on, what makes the ribosomal RNA that is required to get it to the gastrula stage to begin with ?

A. MONROY : These are the ribosomes which are present in the oocyte. Much more information concerning this mutant may be given by Dr. Ebert, because it has been in J. Ebert's laboratory that this work has been done. Would you like, James, to say a little more ?

J. EBERT : Ribosomes are synthesized under the influence of the maternal genome; remember that the anucleate embryos examined result from the union of 2 heterozygotes.

A. MONROY : There was one thing that I failed to say, I am sorry; the anucleolate mutant is obtained by the cross of one-nucleolate heterozygote animals. When you cross two such one-nucleolates (this is a Mendelian character), you get 25 per cent of the animals which are zero-nucleolate.

M. WINICK : Could I also as a matter of information, Dr. Monroy put a question about this amplification : is it DNA replication or is it RNA synthesis from DNA that you are talking about ?

A. MONROY : Yes, I am sorry that I rushed so much. The point is that the section of the genome which codes for the ribosomal RNA and the ribosomal genes are clustered, so that they are much easier to study; they can be isolated and so on. This section of the genome duplicates and produces a huge number of copies of DNA, but this DNA is not directly synthetised on the DNA template. Between the DNA and the DNA replica, there is a RNA in between. That means the DNA synthetises RNA and this RNA acts as template for DNA.

M. WINICK : And what happens to the synthetised DNA ?

A. MONROY : The DNA is trown into the germinal vesicle and then all these

extra copies of DNA start synthetising ribosomal RNA. It is in this way that the egg can cope with the huge demand of ribosomes it has to face in a short time; otherwise it would take some years in spite of the fact that the ribosomal genome is redundant.

Dr. Levinthal is asking me to stress one point which is rather important, namely that in the mammals, at the time of birth, in the ovary you find only oocytes; which means you find cells which are non-replicating. The number of oocytes, that means of future eggs, has already been irrevocably determined before birth. It may decrease, but it certainly cannot increase. And furthermore, at birth, all the events I have just described, that means the synthesis of ribosomal RNA, of the messenger and so on, are already complete at birth. And this again emphasizes the great importance of the prenatal period for controlling what will develop from the individual oocyte.

J. MILLER : Although the challenge of Professor Monroy's observations for the search for teratogens in human beings seems formidables, it is not impossible. The techniques of record linkage which are not appreciated by most people provides a potential tool. The use of this method would permit the long term follow-up of the grand-children of a cohort of women who were exposed to certain agents at the time of pregnancy. Such a technique would produce results only after a long period of time — but my point is it may be the *only* reliable way. I do not believe we can ignore the study of laboratory systems which might provide a faster answer, but I do believe that the grim reality is we cannot ignore epidemiological studies which employ intact human beings as the test system.

C. LEVINTHAL : I don't mean to imply that it couldn't be done, but only that effects of this kind make a purely epidemiological approach significantly more difficult. You need a different kind of data, and as you say, you need linkage which covers a much longer time-span. Furthermore, you could not begin to detect the effect until a great deal of damage had already been done to many people.

A.A. MOSCONA : Of course, our ultimate objective is to understand the situation in the human being. But, perhaps, by using suitable model experimental systems for screening tests we can eliminate some of the inadvertent situations when the human fetus becomes the primary test object for a potential teratogen.

O. HECHTER : In the morning we heard that when human sperm meets up with a human ovum, the possibility of a development error is about 50 per

cent or greater. Now with your sea urchins and your amphibian systems, what would you estimate is the frequency of these developmental defects ?

A. MONROY : Well, as far as I can say, when we fertilise sea urchin eggs, we fertilise them by the millions. The fact is, if you select the proper conditions, or what we call the proper conditions, you get 100 per cent normal development. And yet, there are females, which no matter how accurately you work, how precise you are, in the setting up of the experimental conditions, will not develop properly. That means, the eggs of these females may have, what we say, something wrong although we cannot say what this is. You see, the great problem and the great drawback of the sea urchin egg which, from certain points of view is a very useful material, is that there is no genetics known (our joke in the laboratory is to say that the sea urchins have no genes); not having mutations available, it is inevitable that at one point you find yourself up against a wall. Let me say, for example, that some recent advances in the study of development of the amphibians have been due to the anucleolate mutant and now to the mutant of Humphreys and Brigg (the double 0 mutant). As long as you haven't got mutants, at one point you are in a dead end road.

O. HECHTER : You know, this is a very interesting animal. Without any genetics, but with mutants. But the point is this : if you had a wild strain, a sea urchin egg, or a frog egg, what you are really saying is that fertilisation almost invariably leads to normal development. You have to work very hard to see developmental defects. Isn't that what you said ?

A. MONROY : Yes. But the fact is, out of a few million eggs which are spawned by one sea urchin, I am not sure how many will reach maturity. Natural selection operates at a fantastic high rate : probably one out of a hundred thousand eggs becomes an adult.

O. HECHTER : Can't you reduce the numbers here ?

A. MONROY : Well, I have no figures. I don't think that anybody can quote exact figures.

O. HECHTER : All right, then I want to ask one final question. Given these systems that you have now described, can you add specific chemicals and induce major developmental changes which have been replicated, which are reproducable ? What are the nature of the things you add to these systems which induce developmental changes ?

A. MONROY : Yes, of course. I mentioned actinomycin; and there are chemical means to get two embryos out of one egg by making a certain chemical acting at the two cell stage. There have been a lot of experiments with chemicals which alter development in a well defined way. For instance, two of the most know examples are the experiments of vegetalisation and animalisation : you add lithium chloride and the ectoderm will not develop; the embryo will turn into a huge intestine with a small piece of ectoderm on top of it; or you treat the eggs with zinc chloride and the embryo will never gastrulate and will turn into a ball which is called a permanent blastula. There is a lot of experiments that have been done. What I would agree with you is that most of these experiments are essentially non-interpretable.

T. INGALLS : 1. I would like to ask Dr. Larsson : salicylates in Sweden - how to recognize teratogenesis ?

2. I would also like to ask Dr. McBride : how to recognize thalidomide embryopathy ?

S. LARSSON : We have used salicylates as test compounds to relate the degree of inhibition of acid muccopolysaccharide synthesis and the frequency of different types of skeletal and vessel malformations. More important might be the observations of the different type of fœtal damage if given late in pregnancy to certain strains of mice. These types of " markers " for impairment are fetal liver bleeding, prolonged prothrombine time, reduced liver and heart glycogen content. This is important since the guidelines for new drugs now also includes tests during the last part of gestation, too.

W. MCBRIDE : All pharmaceutical compounds whether new or old can be screened on laboratory rodents, primates, but ultimately *must* be *screened* on human beings, preferably tested first in women where the pregnancy for some reason is to be terminated, so that the fœtus can be thoroughly examined without waiting 9 months in the first instances.

In 1961, we were not using primates, we were using normal laboratory animals which were quite insensitive to thalidomide. I agree with Dr. Miller's saying when he quoted Clark Frazer who in 1963 said that " the ultimate test for any pharmacological compound must be on human beings ". You can screen them on normal laboratory rodents, you can screen them on primates, but you must ultimately test any pharmacological compound, or be it new or be it old, on human beings. And I thing that the work in Sweden where they have been using fetuses, I think that this is one way which you will find quicker than waiting the full nine months.

O. HECHTER : In Dr. Monroy's discussion of the developmental process in very simple systems (e.g., sea urchins, amphibians) he mentioned, in passing, that lithium chloride induces profound abnormalities in development in these very simple systems. One appreciates that such findings in " lower " forms may have no relevance for the problem of congenital malformation in humans. However, this observation may be of some significance; consider the following facts : (a) it is well known that hormone-sensitive mammalian adenylate cyclase systems, which produce cyclic AMP, are profoundly inhibited by lithium; (b) cyclic AMP is now known to play an important role in the regulation of gene activity, both in prokaryotic and eukaryotic cells. These results with lithium thus assume potential significance for teratology. It may be of interest to mention that aspirin, described in passing here as a teratogen, now appears to act *via* inhibition of prostaglandin synthesis; there is, in turn, evidence that prostaglandins may act *via* the influence on adenylate cyclase systems. The specific coupling of lithium and aspirin to cyclic AMP and to gene regulation and development, sketchily developed here, may or may not be significant for the field of teratology. The point I would make is that by study of the " simple " systems described by Dr. Monroy, fundamental principles of embryonic development may be elucidated which provide new insights and important implications for humans.

K. HIRSCHHORN : I would like to open up a very practical method that is really coming to the test very soon and which does deal directly with the human being. Through the work of a number of laboratories, primarily that of Harris in London, a whole series of polymorphisms of enzymes, representing close to about 20 loci, can be relatively easily characterized by electrophoretic systems, from either red cells or white cells or placenta.

If we assume, as I think is legitimate, that at least a certain proportion of teratogenic agents are also mutagenic, then a question of screening for an increase of mutagenic agents in the environment could conceivably come about by first developing a base-line for a mutation rate, utilizing these 20, or hopefully with some more development, 30 ou 40 loci, which can be screened in fetal material in a randomly selected or complete set of births geographically distributed in a predetermined manner.

I known that Jim Neel is devising such a program now with a limited number of markers and hopes to utilize this in Japan to get a base-line mutation rate so that one can periodically repeat such a study in a relatively constant population and see whether one gets changes.

Of course, the way that one would do this is not to look for the common polymorphisms, but to utilize the systems which have been developed electrophoretically, to look for rare variants which have been now demonstrated in

perhaps some 30 loci. If one detects a rare variant, it is relatively simple in most of these to go back into the family and see if in fact this is a new appearance of a rare variant or not. This is basically the approach that can be taken at least to establish a screen for new mutagens in the environment. But first, we need the basic information as to what the back-ground mutation rate is, as done by these markers studies. Also, one very brief and slightly facetious comment; in view of the fact that the vast majority of fertilized sea urchin eggs in fact do not arrive at total development, I was wondering why people were so shocked at the 75 per cent loss of fertilized human eggs.

C. LEVINTHAL : I would like to ask a question of the teratologists : Does anybody have any evidence, one way or the other, either positively or negatively, whether the kind of phenomenon that Dr. Monroy's discussion would suggest in fact exists in higher mammals, that is to say are there third generation effects which are known, are there mature effects ?

M. WINICK : Well, there are third generation effects which have been described with nutritional stimuli, that I can say : I don't know about teratogenic classical agents. But it has been shown by Carli, for example, that if one malnourishes an animal very early in its development, it then goes ahead and, when it shows by the clasiscal psychological testing which are done on rats that this animal does not perform well, if one then mates this rat, then the next generation also shows a dosage effect where it performs less well and it takes until the third generation until the animals are performing normally. So that the question of a third generational effect is certainly there with some of these kinds of experiments.

J. WARKANY : Do teratogens affect the third generation ? The first teratologic experiment done in mammals was by Hale in 1935 who produced eye defects in pigs by vitamin A deficiency. If he made brother-sister matings of such blind pigs (which were fed adequate diets) the third generation young were normal.

T. INGALLS : The experiment I know of that approaches third generation stigmas is the one that was done may be 25 years ago : the Duraswami's work with insulin injection into embryonating chick embryo.

S. LARSSON : Some of our recent studies on cortisone induced cleft palate support the idea of a cytoplasmic factor being responsible for the high frequency of this type of malformation in the A/jax strains of mice. Blastocyst transfer has shown that when the maternal influence could be either uterine or cytoplasmic, the uterine influence is less likely. Thus, when both A/jax

and CBA embryos are growing in their respective foster mothers they show their own full strain characteristics of cortisone induced cleft palate frequence.

P. MARKS : Could you describe in more detail the nature of the studies being performed on human fetuses of mothers anticipating abortions ?

S. LARSSON : We have taken care of the material and preserved it for histological purpose. But some of the material is nowadays being used for pharmacological studies. I think you are concerned about the ethics of this.

P. MARKS : What kind of experiments do you do ?

S. LARSSON : The pharmacologists are testing for instance the receptor functions, the development of the different receptors. Some researchers are studying different steroid hormones at the Laboratory for Reproductive Endocrinology.

P. MARKS : Is this for teratogenesis, in other words is the mother given drugs, who intends to have an abortion ? Is that it ? (answer : yes) And do you have any results ?

S. LARSSON : Yes, you asked that we should study the transfer of different drugs to the mother and this has been done for example with different types of antibiotics, just prior to legal abortions, and I think that also with antiepileptic drugs which have been very much concerned in recent days. It is interesting because in the human fetus it has been found that they can be conjugated in another way than in fetuses of laboratory animals. This is a directly clinical implication, which has to be studied on the human.

J. WARKANY : There was one " human experiment " done with tuberculous mothers in whom therapeutic abortion was tried with aminopterin. Most of these mothers aborted, but a few did not, and in these surgical abortion was performed. The embryos were removed and several were found to be deformed.

W. MCBRIDE : We have been recently studying pharmacological preparations, mainly antibiotics and anaesthetic gases in women having therapeutic abortion, to study the concentrations received by the fœtus.

M. WINICK : Could I just finish a comment that I was making. I am just wondering whether or not the two groups of teratogenists, these two groups

that are emerging, are really asking the same question. In one case, I think that the question that has been asked is : do we need the human to find out if a particular teratogenic agent causes a malformation ? It seems to me that the question the other people are trying to ask is : will a particular teratogenic agent cause a malformation ? I think these questions are very, very different.

If one has to monitor human population to see if any of the agents which are being used are causing malformations, on the other hand, before one puts an agent into use, there is certainly no reason why it can't go through a particular screening test, so that at that point, we can decide whether, or not this particular agent has perhaps a higher potential for being dangerous. So I don't see why the two points of view are incompatible here.

S. BENNETT : I wish to comment again with a little different emphasis. When we speak of the importance of studying the effects of teratogens in humans and of understanding the causes of embryonic malformations as they operate in humans, we are in complete agreement. Let me state also that we will find ourselves unsatisfied if this is all we do. In that event, we shall find ourselves with a large degree of failure in achieving many of the aims which are before us. We shall find that our capacity to improve the lot of manking and reduce the incidents and the devastation of congenital malformations will be improved if we gain more profound understanding of the mechanisms which control development.

Some persons have, in my view, laid insufficient emphasis on the importance of gaining the kind of fundamental understanding.

I wish to emphasize the complementary character of the two approaches; one devoted to study of teratological events in humans, the other on experiments designed to gain basic understanding. In my view, there is no antagonism; each will help the other.

Clinical experiences and clinical observations can be of great inspiration for basic science, as Dr. Larsson has just pointed out in the cases of the recognition of the inborn errors of the metabolism from clinical observations. But at the same time we must state that we would not be able to handle our patients the way we can without our extensive knowledge of genetic control of metabolic pathways, most of which was gained from the study bacteria, yeasts, fungi and the like, and which we then found applicable to man. It was then necessary to gain understanding of the special features operating in man of these more general control mechanisms.

I propose thus to outline the task before us, with its complementary features. In specific operating terms, there may well be a close connection between the malformations in sea urchin development produced by such

experiments as Dr. Monroy reported on the one hand, and human malformations on the other, in the sense that they may represent different kinds of aberrations of control mechanism using the same principles. Dr. Hechter's suggestion proposed that a convergence might be recognized in a common role of cyclic AMP.

In my view, we are not yet in a good position to understand or to formulate in detail the control mechanisms which operate in development, though we can state one or two rather simple things. First, we can say that the general mechanisms of control in cell biology apply also during developmental biology. Thus we can utilize our concepts of messenger-RNA, ribosomes and the like, and put in special factors which can be discovered through research to illustrate how these can affect development in such systems as the sea urchin or the human.

We can also in a somewhat less precise way recall the experiments of Spelmann and of others, the results of which can be formulated succinctly by the statement that in the development of an organism, the development of one component influences importantly the development of a neighbouring component. Yet the mechanisms for these influences are very poorly understood, so that much attention should be applied to the development of our concepts of the control mechanisms of development. As these concepts sharpen, they will find full utility in human teratogenesis and Dr. BcBride and Dr. Ingalls, who are faced with this problem daily with their patients, will be able to deal with their problems more powerfully as these concepts place themselves at their disposal. So, let us encourage and support both basic and clinical approaches and not consider them to be antagonistic.

J. WARKANY: As senior teratologist I shall answer. We are fully aware of the need for cooperation with basic scientists and investigators who work on fundamental embryological and teratological mechanisms. This morning you heard how much we learned from plant and Drosophila cytogenetics and we appreciate the contributions of cytogenetics to a better understanding of some congenital malformations.

The Teratology Society has now been active for 11 years and we always have invited basic scientists to learn from them. If you would read the abstracts of our meetings you would find that many members try to investigate mechanisms but we can still use help. Dr. Bennett, may I extend to you today, an invitation to attend our next meeting, in the hope that you will also make contributions to teratology.

O. HECHTER: Dr. Moscona suggested that it might be possible to differentiate between teratogens in terms of at least five different types of

mechanisms. I would like to ask the teratologists whether an additional mechanism must be considered. We have heard that a very large percentage of the eggs which are fertilized in humans are aborted spontaneously, suggesting that there is an efficient mechanism for rejecting abnormal embryos. The question arises, how does the abnormal embryo signal its presence so that the rejection mechanism becomes operative ? Is it possible that at least some agents which produce malformation, may act by inhibiting the rejection mechanism ? If there is any merit to these questions, the precise definition of this rejection mechanism may well become a critical issue in teratology.

A.E. HELLEGERS : One comment and one question.

The comment 1. — If no one else will, let me question the ethics of giving teratogenic drugs to mothers for purposes of being able to study their effects on the fetus.

The question 2. — I would ask Dr. Winick whether, when third generation nutritional effects are postulated, they operate through the female line and the male line. Is this effect operating through the developing ovary of the female fetus in utero ? I ask since the third generation of the Dutch Hunger Winter will soon be available for study.

M. WINICK : I think the answer that I have to that question might summarize every body's ideas on this point, I don't know the answer to your question whether or not this is paternal or maternal.

W. MCBRIDE : I would like to answer firstly : A court case went on in Germany for nearly three years to decide whether thalidomide acted not by inducing malformations but by preventing the abortion of malformed fetuses, Hellman in London put forward this view, and I think it is quite wrong, I think the Warkany group has proved it to be wrong.

For heaven's sake, don't get the opinion that we are giving women teratogenic drugs, we screen them first on laboratory animals, then on primates. We are testing pharmacological drugs which are available on the market, approved by the FDA, but we still want to know what effect they have on the human fetus and what concentration the fœtus gets.

A.E. HELLEGERS : I wish to make it clear that my comments have nothing to do with the teratogenicity of drugs. I question the ethics of mothers taking any drugs for purposes of having their aborted fetus serve as a useful scientific test substance. Obviously I would equaly question the physician who did the experiment.

J. MILLER : Recently at a research meeting in Vancouver, Dr. Godfrey Oakley of Atlanta, who is working this year with Dr. Tom Shepherd in Seattle, presented data which suggest that women who have received " fertility drugs " have an increased risk of producing offspring with Down's syndrome. I should like to ask Monsieur and Madame Boué whether they have made any observations on spontaneous abortuses from women who were treated with " fertility drugs " ?

J.G. BOUÉ : Nous attendons d'avoir un plus grand nombre d'observations sur les inducteurs de l'ovulation dans le déterminisme des anomalies chromosomiques car il faut faire une comparaison avec des grossesses à terme induites par le même traitement. Notre opinion actuelle est qu'il existe en effet une augmentation des avortements avec anomalies chromosomiques à la suite de traitement par les inducteurs de l'ovulation. Il semble en particulier, que la part de responsabilité est plus grande pour les inducteurs qui agissent directement au niveau de l'ovule (gonadotrophines : HMG, extraites des urines de femmes ménauposées) par rapport aux inducteurs qui agissent au niveau de l'hypothalamus (Clomiphène). Mais ce ne sont que des observations préliminaires.

Journée du 4 décembre 1971

Première séance

STRATÉGIE SCIENTIFIQUE

PRÉSIDENT JAMES D. EBERT

M. MAROIS et G. MAROIS

Méthode histométrique pour tester les anomalies de la différenciation sexuelle somatique

V. INGRAM

Molecular approach of developmental biology

Discussions

Paul MARKS

Molecular approach of developmental biology

Discussions

MÉTHODE HISTOMÉTRIQUE POUR TESTER LES ANOMALIES DE LA DIFFÉRENCIATION SEXUELLE SOMATIQUE

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Les larges applications à la clinique humaine des stéroïdes progestatifs ont suscité l'apparition de molécules nouvelles que le chimiste organicien a livrées au clinicien. Ces stéroïdes peuvent être substitués à la progestérone pour maintenir la gestation en cas de déficience lutéale. Mais leur utilisation chez la femme gestante doit être prudente car certains stéroïdes progestatifs présentent des propriétés secondaires masculinisantes et parfois féminisantes qui peuvent entraîner des malformations fœtales.

Le premier stéroïde de synthèse progestatif, actif par voie buccale, fut préparé en 1938 par Inhoffen et Hohlweg. Il s'agit de la 17-éthinyl testostérone ou éthistérone ou pregnéninolone. Dès 1942, Courrier et Jost attirèrent l'attention sur le danger de l'emploi thérapeutique de cette molécule dans le cas de menace d'avortement. Car cette substance progestative est aussi œstrogène, androgène ; et elle provoque chez la lapine une masculinisation somatique des fœtus femelles et une légère féminisation des fœtus mâles. Cet avertissement ne fut pas entendu jusqu'au moment où des femmes traitées pendant leur grossesse par certains stéroïdes, mirent au monde des filles pseudohermaphrodites.

L'injection d'une hormone sexuelle à la mère gestante retentit sur les fœtus. La démonstration en fut apportée, pour la première fois, par Courrier en 1925 chez le cobaye, et par Courrier et Gros en 1932 chez la chatte, avec la folliculine, tandis que Dantschakoff en 1936 réalisait l'inter-sexualité de l'embryon de cobayes femelles, en administrant du dipropionate de testostérone dans les annexes. Ces recherches princeps furent suivies d'un grand nombre de travaux mettant en œuvre la testostérone, des œstrogènes et, à partir de 1957, de très nombreux progestatifs de synthèse. On trouvera la bibliographie de cette question dans G. Marois (1968 a et b).

Ces travaux avec les hormones mâles et femelles ont permis d'établir les signes morphologiques de la masculinisation et de la féminisation fœtales.

Quelques règles générales ont pu être formulées, les voici : différence de sensibilité des divers récepteurs, importance de l'âge de l'embryon, influence du sexe génétique, différence de réponse du fœtus et de l'adulte à certains stéroïdes de synthèse doués d'activité androgène.

La plupart des recherches précédentes ont mis en œuvre des méthodes histologiques.

Récemment, la modification d'un caractère sexuel externe, la distance ano-génitale (DAG) qui sépare le tubercule génital de l'anus, a été proposée pour apprécier chez le fœtus de rat femelle l'action masculinisante des stéroïdes progestatifs. Cette distance ano-génitale est plus grande chez le mâle que chez la femelle et il est bien établi que les hormones androgènes ou œstrogènes injectées à la mère la modifient.

Nous avons mis au point sur l'embryon de rat une méthode histologique quantitative extrêmement sensible, probablement la plus sensible à notre connaissance, pour explorer le tractus génital, et nous avons confronté ces observations microscopiques avec la mesure de la distance ano-génitale.

Matériel et méthode.

Voici nos expériences : des rattes adultes sont isolées avec des mâles de 18 heures à 9 heures du matin. Nous appelons premier jour de la gestation le jour où la présence de spermatozoïdes est constatée dans le frottis vaginal. Le 14e jour, les mères sont réparties en plusieurs lots :

— mères non traitées, sacrifiées aux 20e, 21e et 22e jours;

— mères traitées par la progestérone, ou l'œstradiol ou le propionate de testostérone, sacrifiées les 22e ou 23e jours;

— mères traitées par un des sept stéroïdes progestatifs :

- trois dérivés de la progestérone :
  l'acétate de 6 alpha-méthyl 17 alpha hydroxyprogestérone ou medroxyprogestérone, la delta-6,6-chloro 16 alpha acetoxyprogestérone ou chlormadinone, la 6-déhydro 6 méthylacétoxyprogestérone ou acétate de mégestrol (*).
- un dérivé de la testostérone :
  la 6 alpha 21 diméthyl 17 alpha éthinyl testostérone ou diméthistérone.

(*) Ce progestatif a été expérimenté en associant un oestrogène dans la même proportion que celle employée en clinique humaine : 0,05 mg d'éthinyl oestradiol pour 4 mg d'acétate de mégestrol.

- deux dérivés de la 19-nortestostérone :
  le 17 alpha éthinyl delta 5-10 œstrène 17 beta ol 3 one ou norethynodrel (*) et la 17-éthinyl 19-nortestostérone ou noréthindrone.
- un dérivé de la desoxy 19 nortestostérone :
  la 3 desoxy delta 4 estrène 17 alpha allyl 17 beta hydroxy 19 nortestostérone, ou allylestrenol.

Le sacrifice de ces animaux a lieu le 22e jour de la gestation.

Les stéroïdes sont dissous dans l'huile et injectés par voie sous-cutanée à raison de deux injections quotidiennes, l'une le matin, l'autre le soir, du 14e au 21e jour. Le sacrifice a lieu le plus souvent le 22e jour.

Les fœtus sont prélevés dans les cornes utérines et pesées; leur distance ano-génitale est mesurée au 1/10e de mm, depuis l'orifice anal jusqu'à la base du tubercule génital; le sexe est vérifié par l'examen macroscopique des gonades. Puis ils sont fixés dans le liquide de Bouin.

Les fœtus sont coupés en série. Les coupes, d'une épaisseur de 5 microns, sont collées et colorées à l'hémalun éosine à raison d'une coupe toutes les 30 coupes, soit tous les 150 microns environ. Tout le tractus génital est examiné, depuis le tubercule génital jusqu'aux gonades.

Nous avons mis au point une méthode quantitative permettant d'établir la longueur des divers organes et leur niveau d'apparition par rapport à la symphyse pubienne et à la vessie.

Schématisation visuelle des résultats histologiques (fig. 1).

Nous avons visualisé ces données quantitatives par des graphiques établis à l'aide des mesures moyennes obtenues à partir de tous les fœtus d'un même lot. Les examens sont toujours pratiqués de la région caudale à la région craniale.

Sur une ligne horizontale représentant l'urètre, les niveaux de référence suivants sont communs au mâle et à la femelle :

— S.B.P. : la dernière coupe où le sillon balano-préputial est visible;
— S.P. : la première coupe où la symphyse pubienne apparaît;
— V. : la première coupe où l'on voit l'urètre s'aboucher à la vessie.

Signalons que la distance symphyse pubienne - vessie du mâle est légèrement supérieure à celle de la femelle.

(*) Ce progestatif a été expérimenté en associant un oestrogène dans la même proportion que celle utilisée en clinique humaine : 98,5 % de norethynodrel pour 1,5 % d'ester méthylique de l'éthinyl oestradiol.

Les organes mâles sont représentés de la manière suivante :

— la prostate (P) par un rectangle dont la longueur est proportionnelle à la longueur réelle de l'organe. Il n'est pas fait mention des répartitions en prostates ventrale, dorsale et latérale, ni du volume de la glande.

— l'utricule prostatique (U.P.) par un petit quadrilatère à l'intérieur du rectangle de la prostate.

— les deux canaux éjaculateurs (C.Ej.) par deux lignes horizontales; ces canaux apparaissent au même niveau que l'utricule prostatique et ils sont prolongés à partir de l'abouchement des vésicules séminales (V.S.) par les canaux de Wolff (C.W.) jusqu'aux testicules droit et gauche (TD; TG). Au niveau où les vésicules séminales et les canaux de Wolff se jettent dans les canaux éjaculateurs, on voit sur les mêmes coupes apparaître les glandes de Cowper (G.C.) (ou glandes bulbo-urétrales).

Les organes femelles sont :

— le bulbe sino-vaginal (BSV) représenté par un étroit rectangle d'une longueur proportionnelle à sa longueur réelle; ce rectangle est prolongé (à partir du clivage (Cl) du sinus uro-génital (S.U.G.)) par le vagin différencié (Vd), puis par le corps utérin (C. Ut.); faisant suite au

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Abréviations utilisées dans les figures 1, 2, 3 *et* 6

S.B.P.	: sillon balano-préputial	♂-♀
S.P.	: symphyse pubienne	
V	: vessie	
P	: prostate	♂
U.P.	: utricule prostatique	
C.Ej.	: canaux éjaculateurs	
C.W.	: canaux de Wolff	
V.S.	: vésicules séminales	
G.C.	: glandes de Cowper	
Tg	: testicule gauche	
Td	: testicule droit	
B.S.V.	: bulbe sino-vaginal	♀
Cl	: clivage	
Vd	: vagin différencié	
C.G.	: canaux de Gartner	
C. Ut.	: corps utérin	
C.M.	: canaux de Müller	
Ov. d	: ovaire droit	
Ov. g	: ovaire gauche	

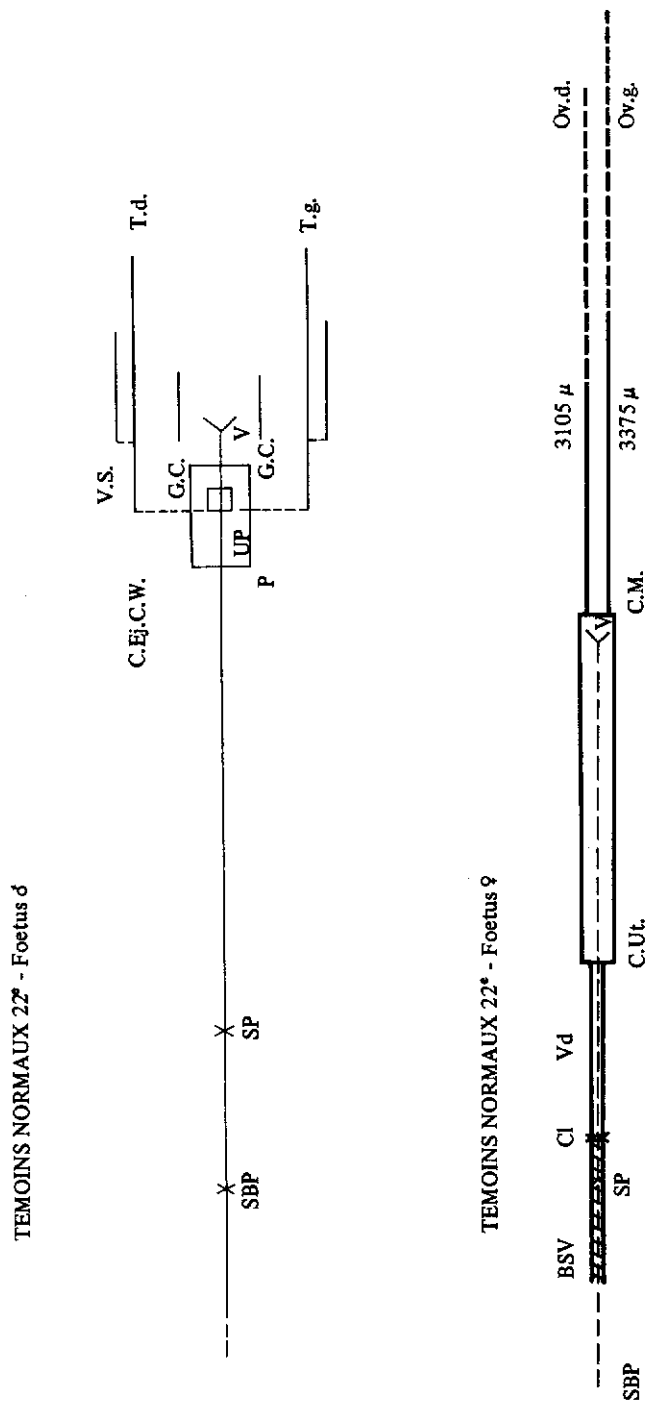


Fig. 1. *Schéma du tractus génital* d'un fœtus mâle (en haut) et femelle (en bas) d'une mère non traitée, sacrifiée le 22e jour de la gestation.

corps utérin, les deux canaux de Müller (C.M.) figurés par deux lignes horizontales. La longueur en microns de chacun des canaux de Müller est mentionnée sur le graphique. A l'extrémité de ces canaux se situent les ovaires droit et gauche (OD; OG).

— les canaux de Gartner (C.G.) vestiges de l'extrémité caudale des canaux de Wolff, sont schématisés par deux lignes horizontales.

Quand les fœtus sont intersexués (fœtus femelles masculinisés ou fœtus mâles féminisés) organes mâles et organes femelles apparaissent sur le même graphique. Il est facile de comparer le tractus génital ainsi modifié au tractus d'un fœtus mâle ou femelle témoin non traité, en superposant les graphiques des témoins reproduits sur papier calque aux graphiques des animaux traités.

Résultats.

1. Tractus génital de fœtus non traités mâles et femelles :

1) La longueur de la distance ano-génitale se stabilise à partir du 21e jour. Elle est plus grande chez le mâle (3,09 mm ± 0,45) que chez la femelle (1,24 mm ± 0,26). Elle est légèrement inférieure le 20e jour, que ce soit chez le mâle (2,46 mm) ou chez la femelle (1 mm).

2) La différenciation sexuelle du tractus génital se poursuit chez le mâle et chez la femelle jusqu'au 22e jour de la gestation. C'est ainsi que le 20e jour, chez le fœtus mâle, la différenciation masculine du tubercule génital n'est pas encore terminée et la prostate n'est encore qu'à l'état d'ébauche. Chez le fœtus femelle, le bulbe sino-vaginal est à peine formé; on observe une fistule uro-génitale et le maintien des canaux de Gartner (ébauches des canaux de Wolff).

Il est important de prendre en considération le développement pondéral du fœtus afin de ne pas interpréter un retard de croissance comme une anomalie de différenciation sexuelle.

Fig. 2. *Schéma du tractus génital.*

— *En haut :* fœtus femelle de mère traitée par 10 mg de testostérone par jour, du 14e au 21e jour de la gestation, sacrifice le 22e jour.

Le développement pondéral est réduit de 24 heures, aussi ce fœtus est-il comparé à un fœtus témoin non traité, sacrifié le 21e jour.

— *En bas :* fœtus mâle de mère non traitée, sacrifice le 21e jour de la grossesse.

Nous avons juxtaposé ces deux fœtus dans la même figure pour montrer que le fœtus femelle, masculinisé, possède en plus des canaux de Muller, et du corps utérin, tous les organes secondaires mâles d'un fœtus mâle de même poids.

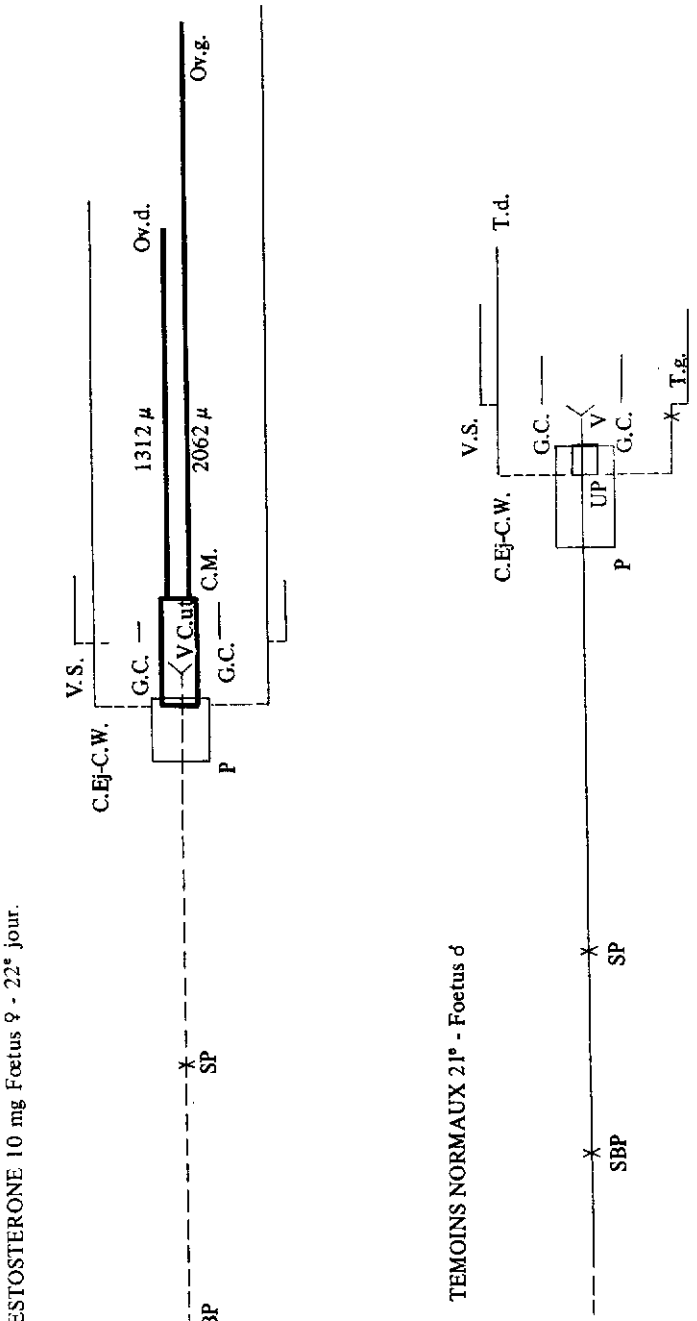

TESTOSTERONE 10 mg Fœtus ♀ - 22e jour.
V.S.
C.Ej-C.W.
G.C.
Ov.d.
1312 μ
V C.ut
C.M.
2062 μ
G.C.
Ov.g.
P
SP
SBP
TEMOINS NORMAUX 21e - Foetus ♂
V.S.
C.Ej-C.W.
G.C.
T.d.
V
UP
P
G.C.
T.g.
SP
SBP

Fig. 2.

2. Action de la progestérone sur le fœtus mâle et femelle :

La progestérone n'a exercé aucune action histologiquement significative, sur la différenciation sexuelle somatique des fœtus de rats mâles et femelles. Les distances anogénitales des fœtus femelles présentent une modification à peine perceptible, dans le sens de la masculinisation. Rappelons que pour certains cliniciens, la progestérone administrée à des femmes au cours de la gestation peut provoquer exceptionnellement une légère virilisation des petites filles à la naissance (Wilkins et alii 1958, Hayles et Nolan 1958, Jones et Wilkins 1960).

3. Masculinisation du fœtus femelle par la testostérone.

Voici la progression de ce processus en fonction de la dose d'hormone mâle (G. Marois 1968 a) (fig. 2) :

1) apparition d'une ébauche de bourgeons prostatiques;

2) puis apparition de glandes de Cowper, très légère augmentation de la distance anogénitale, masculinisation légère du clitoris et totale du sinus urogénital;

3) enfin, masculinisation totale de tout le tractus génital et de la distance anogénitale.

4. Féminisation du fœtus mâle par l'Œstradiol.

Voici l'ordre dans lequel sont atteints les divers récepteurs (G. Marois 1968 a) (fig. 3).

Fig. 3. *Schéma du tractus génital.*

— *En haut :* fœtus mâle de mère traitée par 1 mg d'oestradiol par jour, du 14e au 21e jour de la gestation; sacrifice le 22e jour.

Le développement pondéral est réduit de 24 heures, aussi ce fœtus est-il comparé à deux fœtus témoins, non traités, sacrifiés le 21e jour.

— *Au milieu et en bas :* fœtus mâle et femelle, de mère non traitée, sacrifice le 21e jour de la grossesse.

Nous avons juxtaposé ces trois fœtus dans la même figure pour montrer chez le fœtus mâle féminisé :

— à la différence du fœtus mâle normal, l'absence de prostate et de glandes de Cowper, la position haute du testicule, le plus grand développement de l'utricule prostatique.

— à l'image du fœtus femelle normal, l'apparition d'un bulbe sino-vaginal et le clivage d'une partie du sinus urogénital.

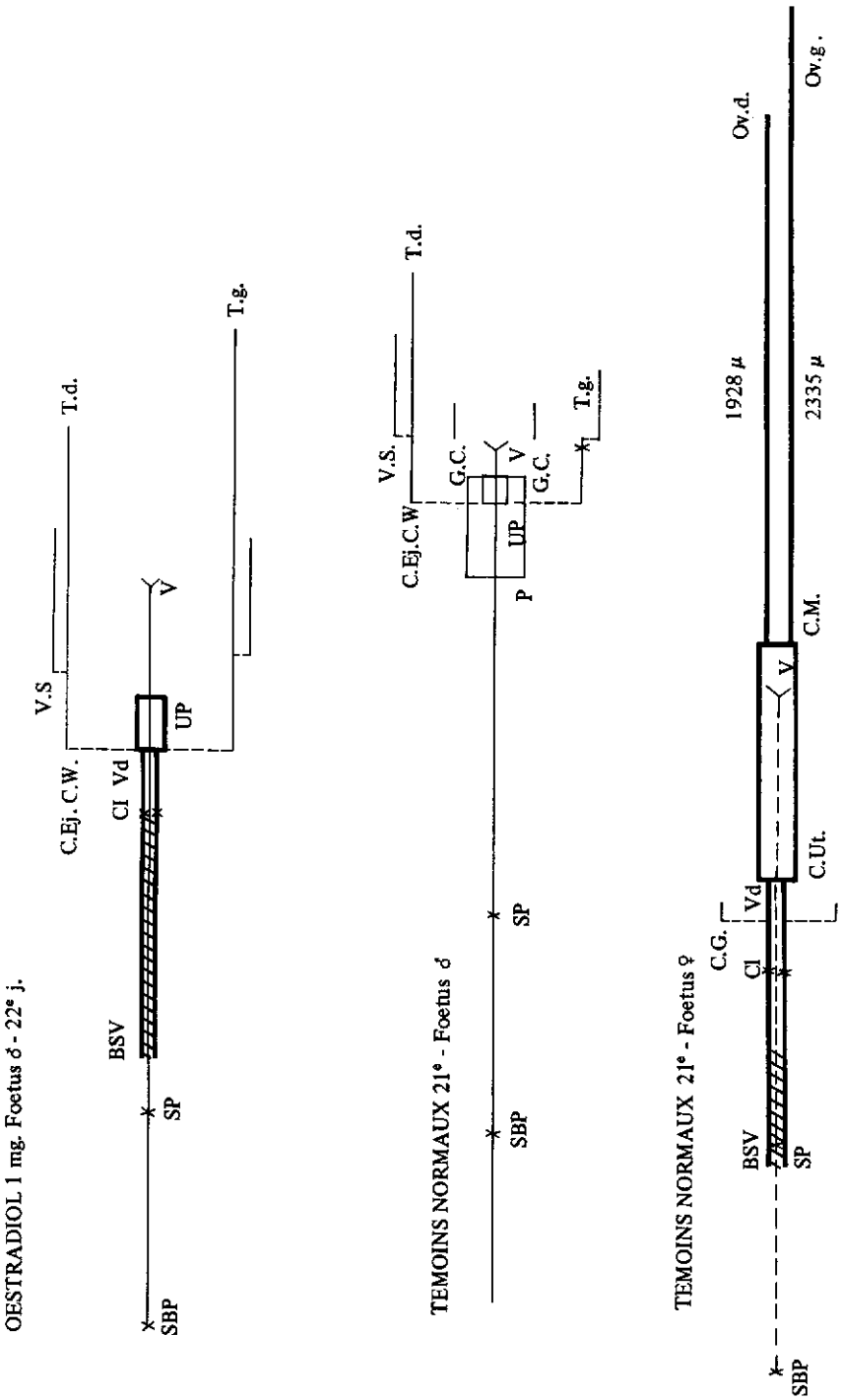

OESTRADIOL 1 mg. Foetus ♂ - 22e j.
V.S
T.d.
T.g.
C.Ej. C.W.
Cl Vd
V
UP
BSV
SP
SBP
TEMOINS NORMAUX 21e - Foetus ♂
V.S.
T.d.
C.Ej.C.W
G.C.
V
G.C.
T.g.
UP
P
SP
SBP
TEMOINS NORMAUX 21e - Foetus ♀
Ov.d.
Ov.g.
1928 μ
2335 μ
C.M.
V
C.Ut.
C.G.
Vd
Cl
BSV
SP
SBP

Fig. 3.

1) De faibles doses provoquent l'inhibition de la prostate (d'abord en volume dans la région ventrale, puis dans les régions dorsale et latérale, enfin en hauteur) et des glandes de Cowper.

2) De plus fortes doses féminisent très fortement tout le tractus génital : féminisation du tubercule génital et de la distance anogénitale; le sinus urogénital court se clive pour former un court vagin; absence de prostate et des glandes de Cowper; les canaux de Wolff sont longs; l'utricule prostatique bas situé est fortement stimulé; les canaux éjaculateurs et les vésicules séminales sont dilatés, les testicules sont haut situés par rapport à la vessie.

Lorsque la féminisation est due à un progestatif, le processus est plus ou moins différent selon le type de stéroïdes.

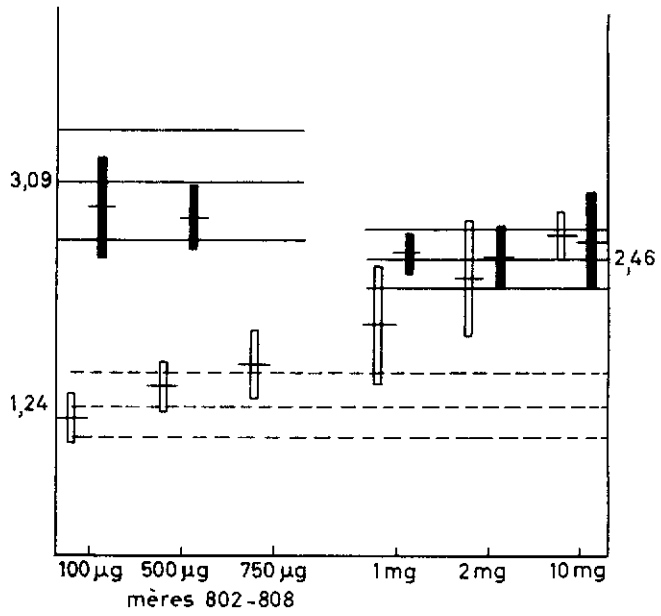


Fig. 4. Moyenne des distances ano-génitales (ordonnée en mm) de fœtus mâles (en noir) et femelles (en trait creux) dont les mères ont été traitées par la testostérone à diverses doses (abscisse). Le sacrifice eut lieu le 22e jour de la grossesse.

Les moyennes de référence des distances ano-génitales ♂ (3,09) et ♀ (1,24) (ligne du milieu) des fœtus témoins non traités prélevés entre le 21e et le 23e jour de la gestation, et les erreurs standard (lignes au-dessus et en-dessous) sont tracées en traits pleins pour les ♂ et en pointillés pour les ♀.

Nous avons mentionné à droite de la figure la moyenne de référence des distances ano-génitales (2,46) de fœtus ♂ prélevés le 20e jour de la gestation.

Valeur du test de la distance anogénitale chez le mâle et chez la femelle.

Dans tous les cas où les mères sont traitées par la testostérone (fig. 4) ou l'œstradiol (fig. 5), une nette modification de la moyenne des distances ano-génitales est un signe infaillible d'atteinte de la différenciation sexuelle du tubercule génital. Lorsque cette moyenne se situe dans la zone intersexuée, toujours le tubercule génital est modifié : soit dans la longueur du frenulum, soit dans la morphologie de l'urètre. La réponse de la distance ano-génitale et les réactions parallèles du tubercule génital sont moins sensibles que les modifications de la prostate et des glandes de Cowper.

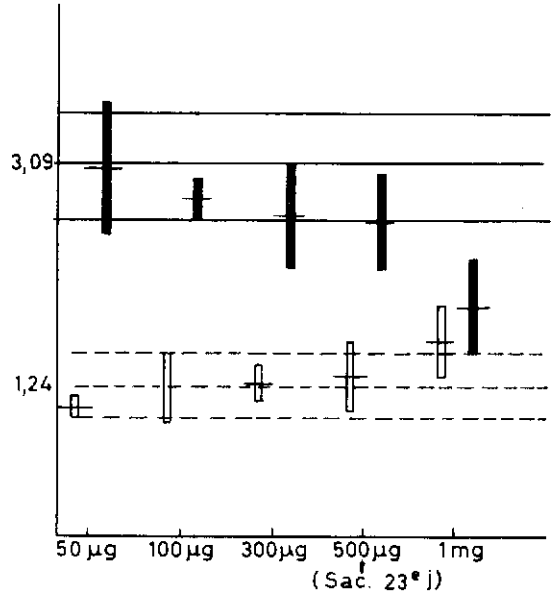


Fig. 5. Moyennes des distances ano-génitales (ordonnée en mm) de fœtus mâles (en noir) et femelles (en trait creux) dont les mères ont été traitées par l'Oestradiol à diverses doses (abscisse). Le sacrifice eut lieu le 22e jour de la grossesse.

Les moyennes de référence des distances ano-génitales ♂ (3,09) et ♀ (1,24) (ligne du milieu) des fœtus témoins non traités prélevés entre le 21e et le 23e jour de la gestation, et les erreurs standard (lignes au-dessus et au-dessous) sont tracées en traités pleins pour les ♂ et en pointillés pour les ♀.

Sensibilité des divers récepteurs sexuels chez le mâle et chez la femelle.

Si nous étudions les divers récepteurs sexuels chez le mâle et chez la femelle, nous constatons que la sensibilité à la testostérone chez la femelle et à l'œstradiol chez le mâle est comparable, en ce qui concerne l'ordre dans lequel ils sont touchés. Pour des doses croissantes, les récepteurs réagissent dans l'ordre suivant :

Sinus uro-génital de la partie haute vers la partie basse : prostate inhibée chez le mâle, induite chez la femelle; bulbe sino-vaginal induit chez le mâle, inhibé chez la femelle et, presque simultanément, modification de l'urètre au niveau du tubercule génital (l'augmentation de la longueur du frenulum précédant la féminisation de l'urètre chez le mâle, la masculinisation de l'urète précédant le rétrécissement du frenulum chez la femelle).

Le territoire des gonoductes réagit en dernier; il est à peine modifié par 1 mg d'œtradiol chez le mâle (allongement et dilatation des canaux éjaculateurs et des vésicules séminales), nettement masculinisé par 10 mg de testostérone chez la femelle (présence du canal de Wolff simultanément avec celle du canal de Muller).

5. Féminisation du fœtus mâle et masculinisation du fœtus femelle par divers stéroïdes progestatifs.

Nos expériences démontrent que la réponse des récepteurs sexuels des fœtus mâles et femelles à des progestatifs doués de propriétés secondaires féminisante et masculinisante n'est pas identique à celle provoquée par un oestrogène pur ou un androgène pur. Elle est différente selon l'importance des deux propriétés secondaires, aussi, avons-nous classé ces stéroïdes en deux catégories :

— masculinisants et légèrement féminisants,

— masculinisants et fortement féminisants.

Nous avons mesuré très exactement le pouvoir masculinisant et féminisant des 7 stéroïdes en recherchant pour chacun les doses liminaires capables de provoquer sur chaque récepteur un début de masculinisation ou de féminisation.

Sensibilité des divers récepteurs sexuels chez le mâle et chez la femelle.

1) Progestatifs doués de fortes propriétés masculinisantes associées à de faibles propriétés féminisantes : c'est le cas de la medroxyprogestérone (dérivé de la progestérone) et de la norethindrone (dérivé de la 19 nortestostérone). Les propriétés masculinisantes l'emportent, quel que soit le sexe, sur toute la longueur du tractus génital.

2) Progestatifs doués de propriétés masculinisantes et féminisantes importantes : c'est le cas de l'association acétate de Megestrol (dérivé de la progestérone) — ethynil-oestradiol (fig. 6) et de l'association Norethynodrel (dérivé de la nortestostérone) — éthynil oestradiol.

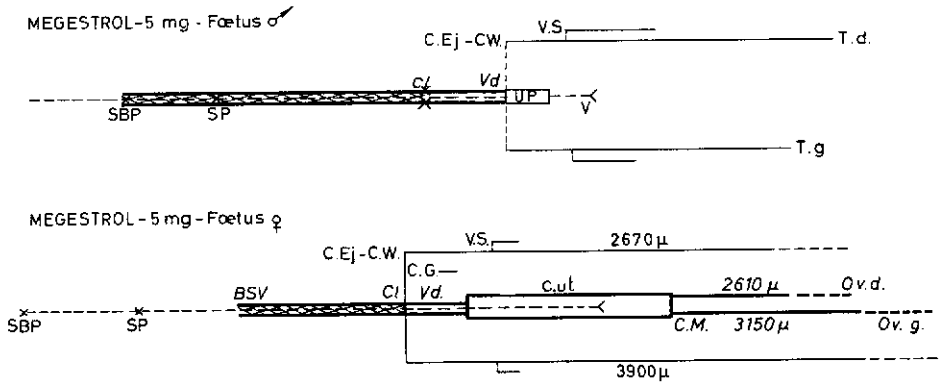


Fig. 6. *Schéma du tractus génital.*

Fœtus mâle (en haut) et femelle (en bas) d'une mère traitée du 14e jour au 21e jour de la gestation par 5 mg par jour de mégestrol associé à l'éthinyl oestradiol (0,05 mg d'éthinyl oestradiol pour 4 mg de mégestrol).

Nous avons juxtaposé ces deux fœtus dans la même figure pour montrer la similitude entre les deux sexes :

— forte féminisation du mâle (comparable à celle du fœtus mâle de la figure 3, féminisé par l'œstradiol),

— masculinisation de la femelle (maintien des canaux de Wolff, apparition des vésicules séminales), sans apparition d'une prostate et de glandes de Cowper à cause de l'inhibition due à la composante œstrogène du mélange.

La réaction des récepteurs est la suivante :

— sur le tubercule génital, les propriétés féminisantes sont prédominantes. En effet, la féminisation pourra être totale chez le fœtus mâle, la masculinisation sera toujours minime chez le fœtus femelle;

— sur le sinus uro-génital, les deux composantes entrent en compétition : dans la partie basse, le sinus est intersexué : bulbe sinovaginal à l'état d'ébauche chez le mâle, mal développé chez la femelle; dans la partie haute se joue l'affrontement : si la composante masculinisante est très forte, on observe chez le mâle, un urètre prostatique de morphologie mâle et le maintien d'une prostate dont le développement (ainsi que celui des glandes de Cowper) est de toutes façons freiné par la composante féminisante : chez la femelle on constate la poussée chez certains fœtus, de bourgeons prostatiques latéraux et d'une ébauche des glandes de Cowper. Si la composante féminisante est très forte, on observe chez le mâle un urètre prostatique intersexué pouvant, chez certains fœtus, se cliver en un vagin court, et l'absence de prostate et de glandes de Cowper; chez la femelle, l'absence de poussée prostatique et de glandes de Cowper est constante.

— le territoire des gonoductes présente toujours des signes de féminisation chez le mâle et de masculinisation chez la femelle, féminisation et masculinisation presque toujours incomplètes (utricule prostatique très rarement stimulé au point de ressembler à un corps utérin chez le mâle; maintien partiel seulement des canaux de Wolff, et apparition seulement chez certains fœtus d'une ou deux vésicules séminales chez la femelle).

Le tubercule génital est donc le territoire le plus sensible à la féminisation. Le sinus urogénital est très sensible aux propriétés féminisantes; toutefois, celles-ci doivent être très importantes pour infléchir la différenciation sexuelle de l'urètre prostatique. Le territoire des gonoductes montre des signes de féminisation chez le fœtus mâle et de masculinisation chez le fœtus femelle.

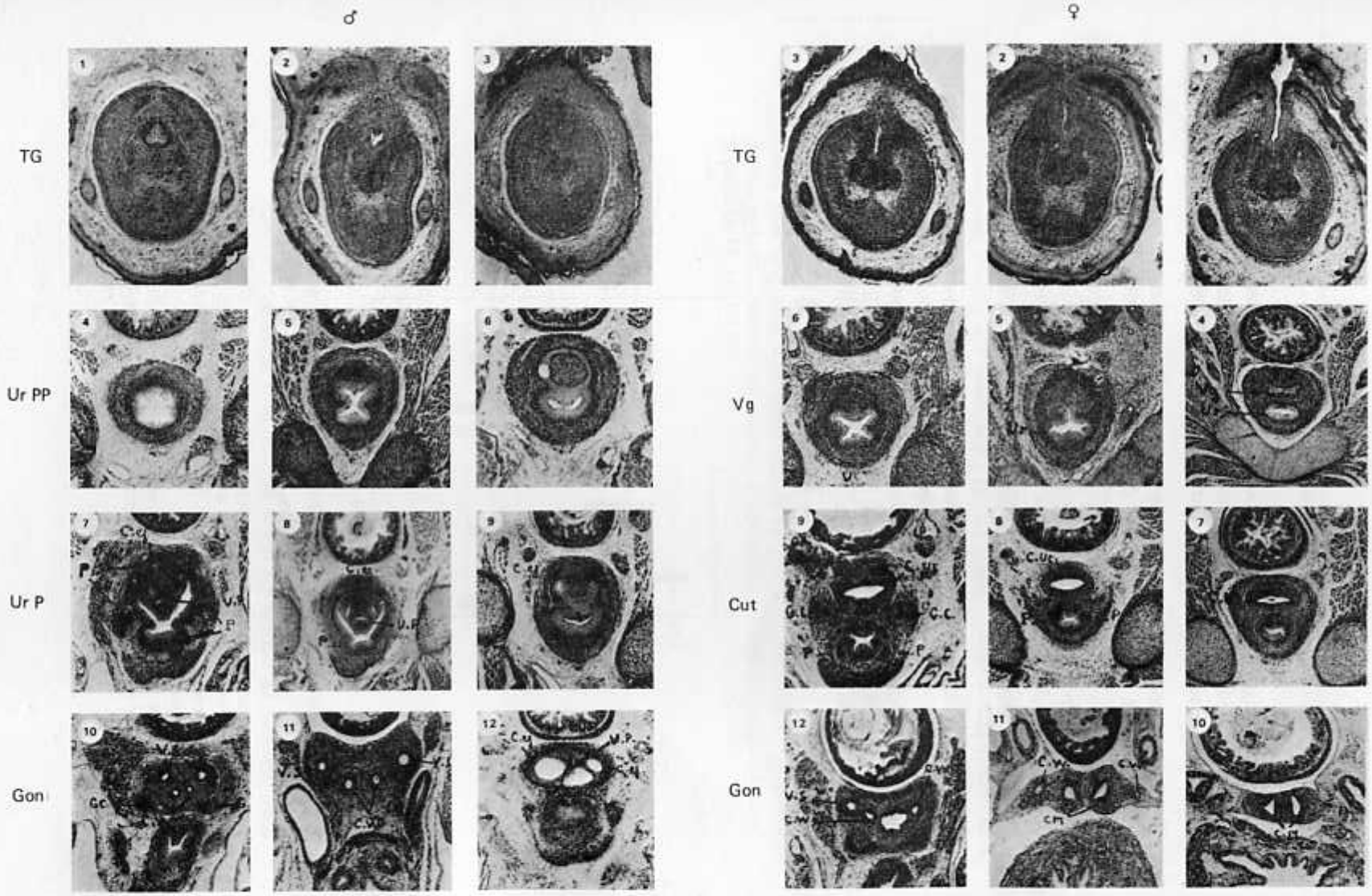
Résumé.

1) Pour apprécier très exactement les modifications de la différenciation sexuelle somatique, leur localisation et leur ampleur, l'examen histologique est la méthode de choix : l'examen de la partie haute du sinus uro-génital décèle

Fig. 7. Tractus génital de fœtus de rats mâles et femelles prélevés au 22e jour de la gestation (planche de gauche : fœtus mâles; planche de droite : fœtus femelles). Progression de la féminisation en fonction des doses croissantes d'oestrogènes, et de la masculinisation en fonction des doses croissantes d'androgènes (il n'est pas fait mention des images de féminisation ou de masculinisation totales).

emplacement des photos	foetus mâles	foetus femelles
colonne de gauche colonne du milieu colonne de droite	image normale légère féminisation nette féminisation	nette masculinisation très légère masculinisation image normale
1ère ligne 2ème ligne 3ème ligne 4ème ligne	T.G. : tubercule génital Ur P.P. : urètre préprostatique Ur P. : urètre prostatique Gon. : gonoductes	T.G. : tubercule génital Vg : vagin C. Ut : corps utérin Gon. : gonoductes

Territoires	féminisation (foetus mâles)	masculinisation (foetus femelles)
Prostate (P) Glandes de Cowper (G.C.) Urètre Tubercule génital (T.G.) Gonoductes (Gon.)	réduction : photo 8 disparition : photo 11 intersexualisation : photos 5 et 9 féminisation : photos 2 & 3 développement de l'utricule prostatique (U.P.) - dilatation des canaux éjaculateurs (C. ej.) : photo 12	apparition : photo 8 apparition : photo 9 intersexualisation : photo 6 masculinisation : photos 2 et 3 maintien du canal de Wolff : photos 11 et 12 apparition des vésicules séminales (V.S.) : photo 12.


♂
♀
TG
Ur PP
Ur P
Gon
TG
Vg
Cut
Gon

les premiers signes de masculinisation : apparition de bourgeons prostatiques latéraux ou, si la composante féminisante s'oppose à cette poussée prostatique de la vésicule séminale formée à partir du canal de Wolff (fig. 7).

2) La mesure de la distance ano-génitale est un critère valable de masculinisation ou de féminisation, dans certaines conditions (fig. 8) :

— chez le fœtus mâle, une diminution des moyennes des distances ano-génitales est un test sensible si le stéroïde a des propriétés secondaires masculinisantes et faiblement féminisantes. Dans tous les autres cas, le test le plus sensible est l'inhibition de la prostate ventrale décelée par l'étude histologique de l'urètre prostatique;

— chez le fœtus femelle, l'augmentation des moyennes des distances ano-génitales est un test valable seulement si la masculinisation est causée par un androgène pur ou par des stéroïdes à composante masculinisante fortement prédominante. Toutefois, ce test est moins sensible que l'examen histologique de la partie haute du sinus uro-génital qui révèle une poussée de bourgeons prostatiques latéraux. Dans tous les autres cas, la mesure de la distance ano-génitale ne permet pas de conclusions.

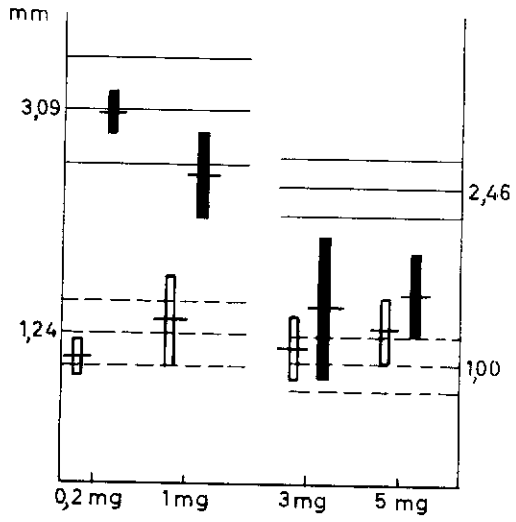


Fig. 8. Moyennes des distances ano-génitales (ordonnée en mm) de fœtus mâles (en noir) et femelles (en trait creux) dont les mères ont été traitées par le *Mégestrol* à diverses doses (abscisse). Le sacrifice eut lieu le 22e jour de la grossesse.

Les moyennes de référence des distances ano-génitales ♂ (3,09) et ♀ (1,24) (ligne du milieu) des fœtus témoins non traités prélevés entre le 21e et le 23e jour de la gestation, et les erreurs standard (lignes au-dessus et au-dessous) sont tracées en traits pleins pour les ♂ et en pointillés pour les ♀.

Nous avons mentionné à droite de la figure les moyennes de référence des distances ano-génitales de fœtus ♂ (2,46) et de fœtus ♀ (1,00) prélevés le 20e jour de la gestation.

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MOLECULAR APPROACH OF DEVELOPMENTAL BIOLOGY

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The influence of molecular biology on development is already undoubtedly very great, as you heard from the elegant presentation of Professor Monroy yesterday afternoon, for example, when he talked about oogenesis. It is quite clear that molecular biology can tell us a great deal about the events of development, particularly the basic molecular events. Within the framework of this meeting, it is surely the molecular basis that we have to understand. This is of course a long-term proposition, there is so much to learn and our ignorance is abysmal. Yet many of us here believe strongly that molecular biology will be able to tell us a great deal about one of the most important problems in developmental biology, namely the difference between the determination of a cell line when a cell changes from being pluripotent to being a uni-or paucipotent cell on the one hand, and further differentiation of a cell-line on the other hand. The understanding of genetic defects, the understanding of all these processes at a molecular level is surely of great importance and, to borrow Dr. Hirschhorn's phrase, nobody is against motherhood, we are all in favour of it, and many of us are in favour of molecular biology.

Now what I can tell you today about molecular biology is surely only a personal experience. In fact, Paul Marks and I, curiously enough, happen to work along very similar lines, namely the development of the erythropoietic system and although he is a « mammal » and I am a « chick », you will see many similarities between us. It is not really possible in the short time available to give a broad overview of the contributions of molecular biology to our field, but there are a couple of ideas which I think I should mention, because they are likely to be of fundamental importance in development.

Quite recently Crick has synthesised the feelings and thoughts of many people on the structure and organisation of the chromosome and he has added ideas of his own to it, as he always does. This model which I will endeavour to draw on the board, it a very simple one. It postulates that the structure of the DNA in the chromosome is composed of two sorts of organisational states, a linear fibrous part, double stranded, interrupted by very considerable globular

portions of no known structure at this point, followed again by another linear portion and another globular portion and so on. What this model does is to emphasise that there are probably two levels of organisation of the genetic material in the chromosome. The level at which the transcription of the genetic code into a message leads ultimately to a protein, such stretches (linear DNA) are repeated. At another level the material (globular DNA) is needed for some function which we don't as yet fully appreciate, which may in part be regulatory, may also be structural in the sense that it keeps the chromosome together.

In relative amounts this model seeks to account for the very large amount of DNA which seems to be present in so many eukaryotic chromosomes, where this globular material might account for some 90 % or so of total DNA. All this, so far, is a synthesis derived from the work and thoughts of many people; the aspect of it which is added is a possible molecular mechanism for regulation of this globular (regulatory) portion of the DNA which says, very simply, that some of the regulatory DNA is open and not double-stranded, and that a regulator substance of whatever nature, positive or negative, might interact not with double-stranded DNA but with regions of single-stranded DNA. Again, this is not entirely a new idea, of course, but it is a very clear statement in Crick's model of this particular idea. So that transcription of the information in that part of the DNA which codes for a protein might begin in the regulatory portion of the DNA and then proceed along the straight part of the DNA.

One further elaboration of this model is the idea that there may be different levels of regulation. I have mentioned the distinction between the event whereby a cell or cell-line becomes determined to a certain pathway of further development and the events which are subsequent to this which are the events of differentiation, the progress along that pathway. Different levels of regulation are to be envisaged.

The process of transcription, the process whereby the information of DNA is transcribed into information in RNA, to which Dr. Monroy referred yesterday, is one in which there has been a great deal of progress in the context of developmental biology. Through the work of groups such as Burgess and Travers we know a great deal about the protein structure of the DNA-dependent RNA polymerase of bacteria and we know of some of the changes which occur in parts of that molecule, a molecule composed of some five different polypeptide chains, some four or five polypeptide chains. We know some of the changes which occur in the structure of this enzyme which are correlated to, or causative in some way of changes in the development of the cell in which they occur. I think, for example ,of the changes which occur in RNA polymerase during sporulation of B. subtilis worked on by Losick

and Sonenshein, where there is a change in development of this micro-organism. They have shown that there is at that point a change in the structure, the primary structure, apparently, of the RNA polymerase : it is a correlation or a causation, we don't know which, at this point.

I think, also, of the work of Rutter and other people, in developing sea urchin embryos, where there are at least three different RNA polymerase enzymes involved. The proportions of these different enzymes changes drastically during the development of the sea urchin embryo. Again, we don't understand the full implications of this observation, but it is the beginning of understanding a vitally important aspect of development at a molecular level, but only a beginning, let me emphasise that.

Now I want to turn to a brief discussion of some of the experiments that we have been doing on the molecular biology of the development of the erythroid system in chick embryos. Our work has been directed towards an understanding at a molecular level and in culture, the biochemical events, which accompany or which cause the changes in differentiation in the morphology of the red blood cells and the changes in the kinds of haemoglobins that are made in the course of development. You can parallel the changes that occur in the intact embryo with the changes with occur in the erythroid cells in culture, at least to some extent, and for a few days. This, in itself, is an important development of technique which many people are now using, that is to say, going from the study of the intact embryo where the problems arise, towards the study of the same events in culture, either in organ culture, or in cell culture. The question immediately arises as to whether what you observe in culture is indeed a representation of what occurs in the intact embryo, and that is the kind of question which you answer according to your taste. Obviously, I like to believe that what we observe in culture mimics what happens in the embryo.

The erythroid system, as you will also hear from Dr. Marks, is well suited to such studies because the erythroid cell is, by its very nature a cell which lives in suspension, free from contact with other cells. It is, therefore, peculiarly easy to adapt to cell-culture conditions, and we do take advantage of this fact. On the other hand, by the time the erythroid cell is circulating in the embryo, all the interesting events are over, it is a largely dead cell which no longer undergoes any cellular or biochemical changes but which merely carries out its oxygen-carrying function. So the events with which we are concerned are the events which precede the establishment of recognisably erythroid red blood cells.

It is fair to say that in molecular biology there are three basic ways in which you try to study the system which you have chosen, having established that it resembles the real-life situation sufficiently. One is straight biochemistry.

You make an extract and you determine what is in it, and what the chemical reactions are. This approach has been very fruitful. Secondly, you can look at mutants which have a step in a sequence of events missing or altered. You compare a mutant with a normal and thereby recognise a step, and having solved what goes on in a step, you can then look at other events in the sequence. Thirdly, you can perturb the system in a number of ways, physically or chemically. The way we have chosen is to perturb our erythroid cells chemically, in particular by adding the drug BUdR, 5-bromodeoxyuridine. This compound is an analogue of a normal component of DNA synthesis, thymidine, and it is incorporated into DNA in place of thymidine. It perturbs developing systems in the following way, and to the extent that it perturbs the system, it has some of the elements of « teratogenesis » in this *in vitro* system. The effect of this drug has been known for about 7 years, through the work of Holtzer originally. If you add BudR to cells which are going to differentiate in culture, such as, for example myoblasts, which are going to form myotubules in culture, if you add it to such myoblasts before they produce myotubules, it will stop that development, but it will not stop cell division. In other words, all the processes which are basic to keeping the cell alive as a cell, and basic to keeping the cell reproducing itself, those, apparently, are not interfered with, but those other processes with allow the cell to make its own special differentiated product, those are stopped. Moreover, the effect can be reversed, as been shown by a number of people, either by removing the drug, or by adding thymidine, the normal analogue of this substance. So it is chemical interference which seems to distinguish between two levels of activity in the cell, namely the normal activity which keeps the cell dividing and the specialised differentiated product-making machinery, or morphology-producing machinery which is characteristic of the cell. Incidentally, there is I think, fairly clearly a threshold effect for this material, if you do a concentration curve.

BUdR works in a number of differentiating systems. It is likely that the effect of this drug, although this is not yet proved, is on cells which are determined but not yet producing the differentiated product.

To introduce the system to you, I have to describe the erythroid cell system in the chick to a certain extent, which I will do as briefly as I can, and this will also serve as an introduction to Dr. Marks' paper.

The essential point I want to bring out is the following, that there are two red-cell lines in those vertebrate embryos that have been looked at, man, mouse, chick, frog. A primitive cell line, which appears early, and a definitive cell line which appears later and eventually is the same as the adult cell line. The primitive cell line is a cohort of cells, that is to say it is a group of cells which appears, differentiates, circulates in the blood to perform its function,

and then goes away. It does not perpetuate itself. Quite different from the definitive cell line, which has a self-perpetuating stem-cell population, from which arise by further differentiating steps the mature definitive erythroid cells. And the two cell lines, at least in the chick, are morphologically different, they are also morphologically different in the mouse. In the mouse they are called yolk-sac cells for the primitive cell line and liver cells for the definitive cell line; liver cells eventually migrate to the marrow, which is where they are formed in the adult. In the chick they are simply called primitive and definitive cell lines.

One other point of interest. Not only are the cell lines morphologically distinct; for example, these mouse yolk cells retain their nucleus even when mature, the mature cells of the definitive cell line in the mouse, like all mammalian red cells, lose their nuclei — not only are they morphologically distinct, but they contain different haemoglobins also. This is true for man, mouse, chick and the frog, the distinction there being between the tadpole and the adult frog.

On the other hand, in the bird and in the mammal, although there are different haemoglobins in the two cell types, there is one peptide chain in common, which for historical reasons we call the alpha chain. However, the morphology and the haemoglobin content are not the only things which change in these red cells in the intact embryo. All this so far is in the intact embryo. My colleague Bernard Moss has been looking at the histone pattern of the red cells, the circulating red cells of progressively older chick embryos. He sees that most of the pattern is really constant, except for one peak, No. 7, which appears just at 4 days. It becomes progressively more important by the time we reach 17 days; in the hatched chick it is about 20 % of all histones. At the same time, all the other histones remain unchanged in absolute amount. This is not a conversion of one histone to another; it is the appearance of a new histone specific for the final maturation stages of these red cells, just before the nucleus becomes inactive. This particular histone has been described before in the literature as fraction V; it seems to be specific for avian erythrocytes.

Well, so much for the *in vivo* situation. Now by taking the very early chick blastoderm at either 20 or 24 hours of development, and disaggregating it mechanically, we can put it into a rotating suspension culture. In that situation the disaggregated cells sort out, the epithelial and fibroblastic cells stick attached to the glass vessel, the erythroid cells remain suspended, so we can get a suspended culture, and we can grow the cells for up to 4 days.

If you plot cell numbers, having done the explantation after 1 day of incubation of the eggs, there is a considerable increase. If one follows any particular experiment, there are between 5 and 7 cell doublings, during the 3 day time period from explantation to day 4; after that the number decreases.

At the same time the cells mature morphologically and one can detect haemoglobin histochemically; by day 4 all the cells are haemoglobinised. Some are quite nicely formed, some are quite heavily vacuolated, but they are all making haemoglobin and they are more or less mature. The haemoglobins from a day 4 culture in gel electrophoresis show the embryonic (E) haemoglobin at the top and the adult (A) and the primitive (P) haemoglobin and the definitive (D) haemoglobin a little bit further down, meaning to say that the cell culture makes all the haemoglobins expected of both the primitive and the definitive red cell series in culture. It makes more of the adult-type haemoglobins A and D than expected from the studies in the embryo, so that the balance between the two cell populations, or the amounts made per cell, in our cultures qualitatively, but not quantitatively ressembles the embryo.

If you measure the cell cycle time by colcemid collection of the mitotic cells, you can show that during the first 30 hours of culture, the cell cycle time is of the order of 11 hours. This corresponds very closely to what Holtzer has measured in the intact embryo. After 30 hours or so, our cells begin to go round the cell cycle more slowly than do the cells in the intact embryo. The point I want to establish is that if we stay in our experiment whithin the first 30 hours or so of culture, we have cell cycle times in culture which quite closely resemble the cell cycle time in the embryo, and it is to these that we can add BUdR, bromodeoxyuridine.

Blastoderms from eggs incubated for 20 hours were taken, put into suspension cell culture and treated with bromodeoxyuridine at 6 micrograms per millilitre, adding the BUdR at different times. If we added it immediately at explanation, then we get the expected cell number increase, but no haemoglobinisation, no haemoglobin formed. In the control, on the other hand, all the cells are haemoglobinised by day 4. So BudR shuts off haemoglobinisation which we take to be the equivalent of differentiation for our purposes. The morphological criteria of cytological staining parallels this. If we add BUdR 5 hours later, we get the same effect. All the cells are sensitive to BUdR. If we add BUdR 10 hours after setting up the culture, however, the cells are no longer sensitive. Well, there is a slight effect, but hardly any effect at all. In other words, the effect of BUdR on these cells is very strongly dependent upon the time at which we add the material. If we use slightly older embryos, the BUdR effect is never complete, something happens between this 5 hour time period here, from 5 to 10 hours after explantation, which we don't yet understand, because after that the cells are insensitive to BUdR. And that is 5 hours in relation to a cell cycle time of 10-11 hours.

If you do the following experiment, and ask — can the BUdR effect be reversed with thymidine ? — the answer is yes. We took 24 hour blastoderms and put them into culture. In the normal course of events they haemoglobinise

almost completely after 3 days, almost all the cells haemoglobinise. If you add BUdR at the time of explantation, there is a very considerable effect, but because these are 24 hour cells, not all the cells are sensitive. The finding is not quite the same as in the previous experiment, about 5 hours off, but these cells have been 4 hours longer in the embryo, the other ones have been for 10 hours in culture, so the parallel is not quite exact. BUdR on cells from 24 hour embryos has a very considerable but not quite complete effect. If, however, one adds thymidine in 12-fold excess at the time of explantation, then the progress of haemoglobinisation is indistinguishable from the control : thymidine by itself is also essentially indistinguishable from the control. So the BUdR effect can be reversed by thymidine at the time of explantation.

What happens if one adds BUdR first and the thymidine later? If one adds BUdR at 24 hours total age and adds thymidine 5 hours later, and then looks on day 4, the BUdR effect is seen to be completely reversible. The same is true a few hours later, if one adds thymidine at 34 hours (10 hours after explanting). However, if one adds thymidine at 45 hours total age, some 20 hours after the explantation started, then one gets a considerably lower degree of reversal, which is probably significant.

The speed of heamoglobinisation after thymidine reversal may be as in the control culture, but it begins 20 hours later. What we are now doing is to use this system to explore the molecular events that occur in these cells when one reverses the BUdR effect, particularly RNA metabolism in the hope that those events will tell us something about the onset of differentiation as defined in these cells.

So, in conclusion let me just say that this is perhaps a brief glimpse, certainly no more, into a particular type of experiment which can be done with a particular developing system. What it will tell us, eventually, about the differentiation and the molecular events of differentiation of that system remains to be seen, and what that, in turn, will tell us of other systems, also remains to be seen, but hopefully will be universally true.

DISCUSSIONS

K. HIRSCHHORN : How is the action of RNAase inhibited ?

V. INGRAM : Oh. Yes. The cells are treated with 0.5 % SDS (sodium dodocel sulphate) and heated to 70 degrees as well for 5 minutes, and then the supernatant from that is applied directly.

L. WOLPERT : Just a minor point. Does BUdR get into DNA ? I ask because this was in some doubt in view of Jacob's results with neuroblastoma which suggested a surface action.

V. INGRAM : We have not yet shown that it goes into DNA of our cells as yet. But, it has been shown very clearly in the hepatoma cells that BUdR enters DNA.

L.T. SAMUELS : The discussion of Dr. INGRAM is related to steroid effects mentioned by Dr. MAROIS. Two periods when abnormalities produced by compounds — when replication is taking place and when differentiation is beginning. Steroids interact with acid proteins of chromatin — is the acidic protein related to regulator area of Crick model ?

V. INGRAM : I think that is very much an open question at the moment, and one which will need a lot of work. It is certainly under very active investigation.

M. WINICK : Two questions : first of all, you are looking at the embryonic protein. Have you looked, or do you plan to look at the mature protein, mature haemoglobin ? And secondly, just a question out of ignorance : are there any haemoglobinopathies in the chick genetically determined which could be, or which you plan to look at in terms of this kind of system ?

V. INGRAM : To answer your second question first : I am not aware of any haemoglobinopathies in the chick that are abnormal. I mean, there are electrophoretically different haemoglobins in certain chick families which could be used as markers. I am not aware of any actual haemoglobinopathies. At the moment we are looking at total haemoglobin synthesis, most of which, in our cell cultures, will be the haemoglobins of the primitive cell line but some of which are certainly also haemoglobin of the definitive cell line. But we haven't yet distinguished between the two. But we will.

M. WINICK : Yes. You're working at such an early time, with a 24 hour blastoderm. How long do you culture for ?

V. INGRAM : We culture for 3 days.

M. WINICK : So that from your first slide it is almost all embryonic.

K.H. DEGENHARDT : I fully agree with your statement that the key to the understanding of mechanisms in teratogenesis is in the molecular level. In this respect I may focuss on the importance of using antimetabolites like BUdR. You mentioned the chemical interference of BUdR with the morphogenetic abilities of the organanlage. Did I understand it well that BUdR makes it possible to differ between the mitotic and the morphogenetic ability in the system ?

I may focuss attention on the experimental work with FCdR which has been performed in our laboratory at Frankfurt. Recently Dr. Fränz stated that FCdR injected in pregnant mice at stage day IX causes a sharp decrease of mitotic rate twenty hours later, which is fully reversed 24 h. later. But in preterm fetuses you state very localized morphogenetic disturbances p.e. in the thoracic vertebral column. So you have both abilities affected, as well the mitotic ability as the morphogenetic potentiality in very circumscribed areas.

So you see, it interferes either with mitotic activity as with morphogenetic activity may be due to spatial level of differentiation in spatial locations. What do you think about this ?

V. INGRAM : I think that is very interesting, and it makes a great deal of sense, because there is another effect of BUdR which I have not mentioned, which is quite well known, but harder to measure. BUdR affects the adhesiveness of cells. Cells which have been treated with BUdR will divide normally, but they are also considerably more flattened and give every morphological appearance of being more adhesive, and surely cell surface properties such as adhesiveness and surface specificity are intimately connected with morphogenetic movements. They must be.

A.A. MOSCONA : Have you had a chance to test the selectivity of the effect of BUdR in your system by measuring the synthesis of other proteins, in addition to that of hemoglobin ?

V. INGRAM : Yes, it is certainly possible. We haven't done anything — our next step is to pursue the RNA and to look at the kind of protein that is being made immediately after reversal, and also compare the BUdR treated with the control groups to ask what kind of proteins are affected.

J. EBERT : Professor Moscona's question is a timely one. I, too, would have hoped that Professor Ingram would have examined the synthesis of other proteins in the erythroblasts in which hemoglobin synthesis is repressed by BUdR. My reason follows : the eukaryotic cell genome contains viral oncogenic sequences, stretches of DNA identical with the DNA of tumorigenic viruses, described by Yoshikawa-Fulcada and myself and confirmed and extended by others. Recent experiments at the National Institutes of Health have revealed that these viral oncogenic sequences (or « oncogenex » as they have been termed by Huebner and his colleagues) can be activated (or brought to expression) by agents such as BUdR, IUdR and methylcholantrene. Viral antigens and other products are produced in mouse cells (in which the oncogenes are normally « silent ») within 48 hours after treatment with these agents.

These findings are significant in themselves, but have a special pertinence to our discussion of the manner in which environmental agents impinge upon the fetus. In teratogenesis as in carcinogenesis, it appears no longer to suffice to consider *only* the environment or *only* the genome, but their interaction. Environmental agents may exert their effects *via* the genome as the foregoing experiments demonstrate most elegantly.

V. INGRAM : Yes, well, I have thought about this a great deal. One of the considerations that has led us to look in other directions first is the apparently very rapid reversal of the BUdR effect, which I find difficult to visualise on the basis of the model of the activation of some protein which is otherwise not present.

A.A. MOSCONA : It might be added here that, as shown by Gordon Tompkins, in hepatoma cell cultures the induction of tyrosine transaminase is blocked by BUdR but the synthesis of several other enzymes is not; thus, in this case, BUdR acts rather selectively.

MOLECULAR APPROACH OF DEVELOPMENTAL BIOLOGY

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As has become apparent during the course of this meeting, our detailed knowledge of the regulation of mammalian cell differentiation, remains limited. Operationally, the definition of the differentiated state generally involves the capacity for the synthesis of specialized proteins. Elucidating the mechanisms controlling the initiation of synthesis of differentiated proteins, requires our ability to study the precursor cell of the differentiated cell. In almost all mammalian cell populations, there is considerable heterogeneity with regard to the stage of differentiation and with regard to the type of the differentiated cell present in any given tissue. In order to identify the initiating event in the onset of the synthesis of differentiated protein of a given cell line, it becomes very important to attempt to isolate this precursor cell so that one may subject it to quantitative, analytical studies. In the total cell population of most differentiating mammalian cell systems, this precursor cell is present in a very small proportion of the total cell population. One of the advantages of the erythroid cell system is that we have been able to make a substantial beginning in the analysis of the aspects of regulation of the biological activity of this precursor cell by approaches which have permitted the isolation of this cell, at least in one system, the fetal mouse which has been used in our laboratory. (For detailed references to the studies discussed below see Marks, P.A. and Rifkind, R.A., 1972). Dr. Ingram has very elegantly shown you that the erythroid cell system has proved to be an extremely useful model for exploring a variety of problems related to the molecular biological approach to differentiation. Some of the advantages of this system include the fact that the erythroid cell system synthesizes a predominant class of proteins, the haemoglobins. In the mature cell, approximately 95 % of all the protein present are haemoglobins. This protein has been extremely well characterized. A cell-free system for protein synthesis has been developed from erythroid cells of a variety of animals which has permitted detailed analysis at the molecular level of events involved in the synthesis of the haemoglobins. The morphology of erythroid cells at different stages of differentiation are well defined and this can be used as a marker for separating stages in the

differentiation of these cells. In addition, in some species, such as the mouse, we have genetics and embryology to take advantage of, in exploring the molecular biological aspect of cell differentiation. There is a hormone, erythropoietin, which appears to induce the differentiation of erythropoiesis from committed cells. I will discuss in some detail the approach to the mechanism of action of this hormone as an example of the molecular biological approach to the effect of specific substances in inducing differentiation. One of the drawbacks of the erythroid system for studies differentiation is the fact that as yet, we have been unsuccessful in cloning these cells in sustained culture. One can maintain these cells in relatively prolonged *in vitro* culture.

Let us turn to a consideration of erythropoiesis in the mouse during fetal development. The mouse has a gestation period of 21 days; the first site of erythropoiesis in the mouse — at 8 days — occurs in the yolk sac blood islands. In this site, cells are produced which synthesize three types of haemoglobins referred to as E-1, E-2 and E-3, (embryonic haemoglobins 1, 2 and 3). These haemoglobins are composed of four separate globin chains : an X chain undistinguishable from that of adult mouse haemoglobin and three embryonic globins, X, Y and Z. Haemoglobin E-1 is composed of alpha and Y chains, E-II, X and Y chains and E-III, alpha and Z chains. The second site of erythropoiesis during fetal development is the liver, and this begins at 11 days of gestation. In the liver, the definitive erythroid cell population develops and these cells produce one type of haemoglobin. This haemoglobin consists of an alpha and beta chain. The haemoglobin produced in the fetal liver erythroid cells is identical to that which is produced in the adult of this species. These cells enter the circulation as non-nucleated reticulocytes. The definitive site of erythropoiesis in the adult of the mouse is the bone marrow.

As you will recall from Dr. Ingram's presentation, this sequence of events is analogous to that which occurs in the chick, in man, and probably in several other species, namely, that during fetal development, there is a change in the pattern of the haemoglobin synthesized which correlates with the substitution of one cell line, in the case of the mouse, liver erythropoiesis, for a primitive cell line, namely yolksac erythropoiesis.

Before proceeding with the details of fetal mouse erythropoiesis, it would be useful to review some of the very considerable advances that have been made in our understanding of the molecular aspects of erythroid cell differentiation. These studies have been carried out in many laboratories in different parts of the world. Based primarily on the work of Bishop and his co-warkers in Scotland, it now appears to be fairly well established that the globin molecules are made of messenger which is transcribed in unique regions of the DNA. There appears to be in the neighborhood of about five globin cistrons per genome or per nucleus.

Messenger RNA for globin has been isolated from the erythroid cells of a number of species — rabbit, man, mouse, duck and several others, in relatively highly purified form. This mRNA can be added to a cell-free system that has been developed from cells that never made globin eg. ascites tumor cells, and direct the synthesis of the globin molecules specific for the cells of origin of mRNA. Work has begun on the structural analysis of the mRNA. A specific initiation transfer RNA is required for the synthesis of globin chain methionyl to RNA. Haemoglobins are synthesized in the cytoplasm on polyribosomes. Polyribosomes which are involved predominantly in the synthesis of alpha chains can be separated from those involved predominantly in the synthesis of beta chains. Possibly three separable factors are required for the initiation of globin synthesis. Under normal conditions, the amounts of alpha and of beta chains, synthesized and released into the cytoplasm, are equal. The mechanism accounting for this balanced synthesis of the two globin chains are not completely understood. The haemoglobin molecule, in addition to consisting of four globin chains, has haem. The synthesis of haem and of globins is also balanced under normal conditions.

In the course of differentiation of at least one erythroid cell line that has been studied in some detail, namely the mouse yolk sac erythroid cell line, division of cells continues for some time after the onset of differentiated protein synthesis. In this cell line ,there is no temporal separation between the synthesis of differentiated proteins and cell replication.

The mechanisms involved in the action of the hormone erythropoietin in inducing differentiation of erythroid cells has been studied in fetal mouse liver erythropoiesis. Central to our understanding of the regulation of erythroid cell differentiation in haemoglobin synthesis, is the identification of the cell in which the synthesis of haemoglobins is initiated.

Erythropoiesis in the fetal mouse liver is a transient phenomenon. It begins on the 10th-11th day. On day 11, 80 % of the erythroid cells present in the liver are at a very primitive stage of differentiation and are classified as proerythroblasts. Over the ensuing 7 days there is, on the average, a 40 to 50 fold increase in the total number of erythroid cells in the liver with a rapid decrease in the proportion of these cells represented by immature cells and a concomitant increase in the more mature haemoglobinised cells, e.g., orthochromic erythroblasts and polychromatophilic erythroblasts. The population of proerythroblasts which is present in the 11 day liver, is metabolically, very active. Proerythroblasts are morphologically indistinguishable on days 11, 12, 13 and 14. However, the population of proerythroblasts present in the 11 day fetal liver, is about 2 to 5 fold more active with respect to both RNA and protein synthesis than morphologically comparable cells at subsequent days of fetal development. This suggests the possibility that these

cells represent the precursor cell population of erythropoiesis in the liver. The disappearance of these cells may be the cellular basis for the transient nature of erythropoiesis in the liver. The proerythroblasts are selectively responsive to the hormone erythropoietin.

There is considerable evidence that the so-called stem cell is a pluripotential cell which can differentiate to erythroid cells, white cells, platelets and other large macrophages. The stem cell is distinct from the immediate percursor of the erythroid cell. One can separate these cells by physical means, for example, by centrifugation. The erythroid cell precursor, then, is a cell which is determined but which is not yet expressing haemoglobin formation. Goldwasser and co-worekrs and Paul and co-workers have shown that the hormone erythropoietin, when added to rat marrow or fetal liver respectively, will markedly increase the amount of haemoglobin production.

The stimulation in haemoglobin formation induced by erythropoietin does not reflect the effect of the hormone on haemoglobin formation per cell. The hormone does increase the number of cells which are involved in haemoglobin synthesis. Addition of erythropoietin to a population of erythroid cells removed from fetal liver at day 12 or 13, results in a marked increase in the total number of cells, a maintenance in the population of the early erythroid cells capable of cell division, and an increase in differentiated, non-nucleated, haemoglobin containing cells.

Thus, this hormone is working to affect the maintenance of the immature cells in the population, cells which are capable of continued division and differentiation to the cell types which will synthesize haemoglobin.

To more carefully examine the nature of the effect of this hormone, we turned to study the question as to the type of cell which responds to erythropoietin. One marker for responsiveness to erythropoietin is an early stimulation of RNA synthesis. It could be shown that erythropoietin was highly selective in its effect. Erythropoietin selectively stimulates uridine uptake and incorporation to RNA in the earliest identifiable erythroid cell precursor, namely the proerythroblast. The hormone neither effects RNA synthesis at later stages of differentiation of erythroid cells nor does the hormone have any effect on non-erythroid cell elements. In other words, these data are consistent with the concept that the hormone acts on an erythroid cell precursor which is itself differentiated in the sense that it is determined. The hormone sets off the process of differentiation. This effect of the hormone in stimulating RNA synthesis does not require any preceding effect on DNA synthesis.

In fact, it is some 11 to 12 hours before there is detectable effects of the hormone on DNA synthesis while stimulatin of RNA formation occurs

within 1 hour. It requires 12 to 18 hours before there is a measurable increase in haemoglobin formation in these experiments.

With the recognition of the selective action of erythropoietin on the earliest erythroid cell, it became desirable to isolate this cell, so that it could be studied in more detail. Richard Rifkind and his co-workers developed a technique for the isolation of these cells which involved the use of an antibody to the adult mouse reticulocytes. This antibody, in the presence of complement, will selectively lyse all but the earliest proerythroblasts. Employing this procedure, one can obtain a population of cells which are almost uniformly proerythroblasts and basophilic erythroblasts. These cells can now be placed in culture and require, for survival, the addition of erythropoietin. Within 6 hours of culture without erythropoietin almost none of these cells remain viable. By contrast, in the presence of erythropoietin, the immature erythroblasts will multiply and differentiate to mature non-nucleated erythroid cells synthesizing haemoglobin over a period of 48 to 72 hours.

The proerythroblast is a very large cell, with a large nuclear cytoplasmic ratio, a large nucleolus and cytoplasm which contains abundant polyribosome. On the basis of the date summarized above, our present understanding of the mechanism of erythropoietin action is as follows : the pluripotential cell is presumed to be a precursor of the erythropoietin-responsive cell (ERC). The ERC is itself differentiated in that it has developed a recognition mechanism for the hormone. The hormone acts selectively on this cell and the initial effect on macromolecular synthesis is an increase in RNA formation. The RNA in turn leads to an increase in DNA synthesis, mitosis and the differentiation of erythroblasts to synthesize haemoglobin. It is of interest that a number of hormones which appear to induce cell differentiation act by initially stimulating RNA synthesis and cell replication.

In the mouse there are certain interesting mutants which offer possibilities for exploring further the mechanism involved in the hormone controlled differentiation of erythroid cells. We owe most of our knowledge with respect to these mutants to the work of Elizabeth Russell and her colleagues at the Jackson Laboratories in Bar Harbour. It is through her kindness that these mutants have been made available to us for study. There is a series of mutants referred to as W mutants, all of which have severe anemias. At least 5 W mutants have been distinguished, in which the defect appears to involve a lack of responsiveness of the erythroid cell precursor to erythropoietin. Animals which are doubly heterozygous for two of the mutant types may survive, with a survival rate of about 60 %. These animals homozygous for a lethal W mutant gene can be " cured " by injection of erythroid cell precursors from a wild type. However, the precursor cells from W mutants injected into irradiated normal donors will not proliferate in the normal recipients.

There is another type of mutant referred to as Steel, or S1 in which the defect in erythropoiesis seems to involve some environmental factor. Here the precursor cells can be removed from the S1 fetal livers, injected into irradiated recipients and develop normally. One can also demonstrate, in contrast to the W mutants, that these erythroid cell precursors will respond to the addition of erythropoietin *in vitro*. In other words, the mutation appears to involve a lack of responsiveness to erythropoietin, while the S1 mutants seem to have an environmental defect which prevents the erythroid cells from responding to the hormone.

There are two other mutants which manifest themselves by severe anemia and inhibition in the development of fetal erythropoiesis but essentially no studies have been done to analyze the level of the defect in erythroid cell development. There is a 5th mutant which is of considerable interest and studies on these are in their embryonic stage but this is a mutation which involves the failure of development of the first erythroid cell line, i.e., yolksac erythropoiesis. This is not associated with any impairment in fetal development, as far as one can tell, and these animals, when born, look grossly normal.

In summary, the definition of the molecular basis of the control of cell differentiation can be significantly facilitated if one can work with isolated cells which are precursors of a differentiated cell line. In the metal mouse erythroid cell system we have a model which lends itself to considerable detailed analysis of the normal mechanisms of differentiation. This model should be useful in the analysis of a variety of substances which can alter normal differentiation of mammalian cells.

Reference.

P.A. Marks and R.A. Rifkind, Protein Synthesis : Its Control in Erythropoiesis. *Science*, **175**, 955 (1972).

DISCUSSIONS

J. EBERT : Thank you, Paul. Dr. Marks' paper is now open for your comments and questions.

C. LEVINTHAL : Two questions. I justdon't know eneugh about the biology of this, but what is known about the control of the production of the enzyme, of the hormone in the normal system, and are there any temperature sensitives in any of these classes of mutants of Russel's ?

P. MARKS : I can answer the second one very easily. I don't know of any temperature sensitive mutants in the erythroid cell system. A good deal is known about the physiological control of erythropoietin. It is a hormone that is presumed to be primarily produced in the kidney. Erythropoietin production is sensitive to anoxia. Under conditions in which tissue anoxia is produced, there is a stimulation in the production of erythropoietin. Under conditions of anemia, there is increased erythropoietin production; under conditions of polycythemia, there is a decreased erythropoietin production.

V. INGRAM : 1) What's the effect of erythropoietin on yolk sac and on liver erythroid cells. 2) What's answer about erythropoietin production in the fetus ?

P. MARKS : Erythropoietin has no demonstrable effect on the yolksac erythroid cells. This is work both from John Paul's laboratory and our own. The question of erythropoietin production in the fetus is one that is under intensive examination. The best evidence probably derives from Gordon's laboratoiry, where he feels that he has evidence that there is erythropoietin production in the fetus which begins at about the same time as the onset of liver erythropoiesis, namely about the 11th day of gestation in the mouse. He has some experiments to suggest that fetal anoxia is associated with increased fetal erythropoietin. There is a controversy in the literature as to whether maternal erythropoietin gets across the placental membrane. Since erythropoietin has not been purified, direct experiments have not been possible. On the basis of biological studies, the balance of the evidence would be, I think, against maternal erythropoietin crossing the fetal membrane.

M. WINICK : Does DNA synthesis have to take place for the action of erythropoietin and if not are the two factors separable temporally in time ?

P. MARKS : Correct. I think in the erythropoietin fetal mouse system there is no question that the hormone will induce RNA synthesis prior to detectable effection DNA, as well as in the presence of hydroxy urea or cytosine arabinoside which inhibit DNA synthesis approximately 95 %. I would have to emphasize that cell replication does not occur nor does hormone-stimulated haemoglobin synthesis occur if DNA synthesis is blocked. If there was a hormone stimulation of haemoglobin synthesis per cell, as a result of the RNA synthesis prior to cell replication, we may not be able to analytically detect it .That the differentiation of the committed cell involves a requirement for at least one mitosis, is a possibility, on the basis of our data.

K. HIRSCHHORN : Using lymphoid systems o,ne can perhaps develop a testable rule. Stimulation of lymphocytes causes an increase in the synthesis of pre-existing molecules, e.g. migration inhibitory factor and lysosomal enzymes, which is unaffected by inhibition of DNA synthesis. However, primary sensitization to antigen in vitro (Feldman, Israel) requires one and only one cell division for new antibody synthesis. The rule to be tested is :

1) synthesis of products of preexisting messages is by amplification;
2) production (induction) of new message requires event of DNA synthesis.

V. INGRAM : Are you sure that it isn't just maintenance, during the few hours when you are measuring whatever synthesis you are measuring ? I mean as opposed to the synthesis of new products ? Conceivably this might be the situation in erythropoietin.

D. HSIA : How specific is the effect of erythropoietin in terms of source and response of species of mammals ?

P. MARKS : There seems to be no species specificity with respect to the origin of erythropoietin among mammalian species. The erythropoietin that we now routinely use is of human source. The response qualitatively and quantitatively to mouse erythropoietin is indistinguishable from the human.

A.E. HELLEGERS : I wonder if you could expand a little further on the evidence against erythropoietin crossing the placenta. I could think of some evidence in favor, but short of molecular size perhaps, I didn't know of any evidence against.

P. MARKS : I said the evidence is not good. In other words, not direct. Jacobson, for example, has done biological experiments of the following nature. He has induced hyper-erythropoietinemia in a pregnant animal, either by

injecting erythropoietin or by bleeding the mother. Under such circumstances there is no detectable effect on red cell production in the fetus. Jacobson has also done the reverse experiment. He has induced polycythemia in the pregnant female, suppressed erythropoiesis in this manner in the mother, and observed no effect on fetal erythropoiesis. These data suggest that maternal erythropoietin is not having any biological effect on the fetus. I wonder if others know of any better data.

B.C. GOODWIN : Is there any evidence for the existence of factors which inhibit recruitment of cells into the erythroblast system so that there is the possibility of clinically controlling polycythaemia by a mechanism other than via erythropoeitin ?

P. MARKS : Evidence with regard to these factors is at a rudimentary level. There is some evidennce, for example, for factors which appear to be associated with white cells, in other words, produced by white cells, which will specifically inhibit erythropoiesis and the action seems to be possibly competitive with erythropoietin. The nature of these substances has not been defined. One of the intriguing posibilities here is the possibility of analyzing the determinants of the direction in which pluripotential cells will go. There must be some factor or factors that are controlling at this level.

Journée du 4 décembre 1971

Seconde séance

STRATÉGIE INSTITUTIONNELLE

PRÉSIDENT JAMES D. EBERT

J. FOURASTIÉ

Quelques considérations économiques

Discussions

Conclusions

QUELQUES CONSIDÉRATIONS ÉCONOMIQUES

J. FOURASTIÉ
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C'est un grand honneur pour moi de participer aux travaux de votre fondation, étant donné l'importance humaine et sociale des questions que vous avez envisagées ici. M. Marois m'a demandé de dire quelques mots sur les aspects économiques du sujet. Il est comme moi-même, tout-à-fait conscient du fait que ces aspects économiques sont beaucoup moins importants, et je dirais même dérisoires, à côté des préoccupation humaines, des problèmes de souffrance, de douleur qu'entraîne l'inadaptation.

Néanmoins, il est clair que dans nos civilisations les notions les plus abstraites et la souffrance elle-même ont un aspect économique et entraînent des problèmes financiers. On voit bien pourquoi : ce n'est pas seulement un abus d'une certaine forme de société, c'est que tout soin apporté par un être humain à un autre humain implique du temps passé, implique du *travail,* et ce travail a un coût économique. Il faut des travaux humains pour prévenir les malformations ou les inadaptations; il faut aussi du travail humain pour atténuer les conséquences de la malformation ou de l'inadaptation.

Par conséquent, il y a un coût de la prévention, et il y a un coût des soins de la maladie une fois existante, de la malformation une fois constatée. M. Marois m'a demandé de poser le problème devant vous et d'essayer d'évaluer d'une manière quantitative ce volume de travaux et par conséquent, ce volume de monnaie. Je dois le dire, la question m'a été soumise il y a relativement très peu de temps; j'ai fait un tour d'horizon : comme je l'avais pressenti, j'ai trouvé des problèmes posés beaucoup plus que des solutions ou que des informations. J'ai fait rechercher s'il y avait par exemple, des livres sur le sujet : il y en a quelques-uns, bien sûr mais aucun qui traite directement le problème. Les informations sont rares, elles sont sporadiques, décousues et partielles.

Quant au coût de la prévention, je ne dirai rien, puisque la prévention à l'heure actuelle ne vas pas très loin et que, justement, vous envisagez des techniques nouvelles qui pourraient être efficaces; mais il faut d'abord définir ces techniques pour en apprécier la valeur en travail humain et la valeur, disons,

en francs ou en dollars. Mon enquête du côté prévention, visites pré-natales, etc., ne m'a rien donné de précis puisque, en somme, ces démarches sont communes à toute une série d'autres qui ont des objectifs tout différents. En conséquence, sur ce point-là, je ne vois aucun chiffre qui vaille la peine d'être donné et qui puisse permettre de savoir ce que coûtera cette prévention encore à l'état de projet. Le coût dépendra beaucoup des procédures qui devront être utilisées et à l'heure actuelle, à ma connaissance, du moins en France et dans les pays que j'ai étudiés, il n'y a pratiquement pas de prévention efficace dans le domaine dont nous parlons.

Quant au coût social de l'inadaptation, j'ai pris cette notion dans le sens où on la prend en France, parce que je ne savais pas très bien comment la prendre autrement, et vous le savez, c'est une notion assez floue; on y range énormément de catégories différentes de gens, depuis ceux que nous appelons en France les « inadaptés » proprement dits, jusqu'à ceux que nous appelons les « débiles légers », en passant par des quantités de catégories du genre « infirmes moteurs cérébraux, infirmes moteurs non-cérébraux », etc. Ce que j'ai retenu, c'est que vous vous préoccupiez des handicaps dont l'origine est biologique et non pas des handicaps qui sont nés d'accidents du travail ou d'accidents de la circulation.

Dans cette grande plage, je peux vous apporter deux chiffres qui paraissent essentiels pour envisager le coût social de l'entretien des enfants handicapés. C'est d'abord pour la France, une indication du nombre des personnes présentant un handicap d'origine biologique, allant des débiles légers aux handicapés totaux. J'ai sous les yeux un état qui envisage que leur nombre est en France de 938 000 (tableau 1). Une autre source — il ne faut pas s'étonner qu'elle diverge — donne un chiffre nettement plus faible de l'ordre de 700 000. La différence

TABLEAU 1

Effectif des handicapés mineurs jusqu'à 20 *ans* (*)

Débiles légers	369 000
Caractériels débiles légers avec troubles associés	67 000
Débiles moyens	123 000
Débiles profonds	92 000
Arriérés profonds	30 000
Infirmes moteurs cérébraux	22 000
Infirmes moteurs non cérébraux	123 000
Aveugles et amblyopes	16 000
Sourds et mal entendants	11 000
Infirmes et handicapés de 0 à 5 ans	85 000
	938 000

(*) Rapport de la commission d'Action Sociale du 6e Plan.

TABLEAU 2 (*)

1967 : Capacité d'accueil des établissements spécialisés

Enfants et adolescents handicapés et inadaptés.	
handicapés mentaux :	
légers	128 000
moyens	20 000
profonds	13 000
arriérés profonds	7 500
	168 500
handicapés physiques :	
moteurs	6 000
sensoriels	11 000
	17 000
troubles du comportement	29 000
	214 500
enfants et adolescents menacés d'inadaptation	73 000
Adultes handicapés ou inadaptés.	
handicapés mentaux :	
hôpitaux psychiatriques	112 000
autres établissements	2 000
	114 000
handicapés physiques	10 000
inadaptés sociaux	100 000
détenus	31 000
	255 000
Total général	542 500
	(1 % population)

(*) Extrait de Michel Euvrard : *Etude du problème général de l'inadaptation des personnes handicapées.* Annexe E : examen de quelques aspects financiers, 1967.

TABLEAU 3 (*)

Recensement 1962.

1 690 000 personnes se sont déclarées atteintes d'infirmités ou d'autres incapacités physiques permanentes (3 % de la population).

Infirmités chez les hommes : — 30 % dues à la guerre;
— 29 % dues à des accidents de travail.

La plupart des infirmes sont du sexe masculin : 1 190 000 sur 1 690 000 (71 %).

Infirmes mentaux : 73 000 dont 38 000 sont dans des établissements de soins.

1967.

Régime	
Sécurité Sociale :	
invalides « ordinaires »	51 537
accidents du travail	123 320
	174 857
Aide sociale :	
infirmes	22 000
grands infirmes	255 000
mineurs surveillés	107 000
Guerre :	
invalides	1 658 000
	2 042 000
Enseignement public :	
classes spécialisées	140 000

Ordre de grandeur du nombre des handicapés en 1967 : 2 à 3 millions de personnes.
Pour les inadaptés, aucune information, mais leur nombre ne doit pas être inférieur à 600 000.

(*) Extrait de Michel Euvrard : *Etude du problème général de l'inadaptation des personnes handicapées.* Annexe E : examen de quelques aspects financiers, 1967.

tient avant tout au nombre des débiles légers : dans la dernière statistique, on a compté comme normaux une grande partie d'entre eux; dans l'autre statistique, on les chiffre à 370 000; donc il s'agit d'un gros bloc; selon la manière dont on traite ce bloc, on a des chiffres très différents (*).

(*) Les tableaux 2 et 3 ci-dessus fournissent quelques informations complémentaires.

De toute manière il s'agit de chiffres importants; un homme sur cinquante donc, serait dans un état de handicap du fait de sa biologie, si je puis dire. Si nous arrivions à éviter leur naissance, soit en faisant qu'ils ne soient pas conçus, soit en faisant qu'étant conçus, ils deviennent normaux, ce serait un résultat qui, au point de vue démographique, porterait sur des nombres importants : 2 pour cent de la population.

Voici maintenant quelques informations elles-mêmes grossières, elles-mêmes sujettes à caution et à discussion sur le coût d'entretien, par jour, ou par vie, de ces inadaptés. Ici, je crains que le mot inadapté n'ait pas toujours été pris dans le même sens que précédemment; voilà qui rend très difficile l'étude du sujet; le mot « inadapté », est un mot vague et les médecins, les statisticiens, les sociologues ou les administrateurs, l'emploient dans des sens très différents. Je ne peux donc pas garantir que lorsque je vais parler maintenant de l'inadapté moyen, il s'agisse effectivement de l'inadapté moyen défini comme précédemment, c'est-à-dire allant du débile léger en nombre de 370 000 aux handicapés les plus graves qui sont de l'ordre de 150 000 ou 200 000.

Voici les chiffres que j'ai obtenus auprès d'une part de la Sécurité Sociale française, d'autre part, auprès de différents spécialistes qui ont étudié la question; notamment le CREDOC a fait des enquêtes sérieuses sur le sujet (le CREDOC est un Centre français de Recherches et de Documentation sur la Consommation, sur la consommation des familles au sens très large du terme. Et la consommation des familles, ce n'est pas seulement du pain et du chocolat, ce sont des services médicaux et ce sont aussi des dépenses pour les enfants inadaptés). Le CREDOC, institution subventionnée par l'Etat, travaille en liaison étroite avec les services officiels, avec l'Assistance Publique, avec la Sécurité Sociale, avec l'Institut National de la Statistique. Donc il s'agit de chiffres sur lesquels on peut compter. Le CREDOC a fait des études comparées sur ce que coûte dans une même famille un enfant normal par rapport à un enfant inadapté.

Les écarts sont vraiment considérables. L'enquête est de 1967; un enfant inadapté coûte en moyenne 27 francs par jour, en 1967, — donc environ 5 dollars — pour l'entretien et aussi, l'éducation. Par contre un enfant normal coûte 4,50 francs, c'est-à-dire moins d'un dollar. Il s'agit d'enfants de moins de dix ans. L'écart serait donc de un à cinq; l'enfant inadapté moyen coûterait en moyenne à sa famille cinq fois plus cher que l'enfant normal (tableau 4).

L'autre chiffre m'a paru intéressant pour fixer quelques ordres de grandeur. Il se réfère non plus au coût journalier de l'entretien et de l'éducation d'un enfant, mais au coût dans la vie entière d'une personne inadaptée, coût de la prise encharge par la société (c'est-à-dire ce que la société dans son ensemble devrait apporter à chaque inadapté pour combler son déficit économique et

TABLEAU 4

Enquête CREDOC (1967)

enfants	coût annuel moyen
1	1 700 F
2	3 200 F
3	4 500 F

Eléments du coût d'un enfant	normal	inadapté	écart
1 seul enfant :			
coût brut	4,7	27,0	22,3
prestations familiales	0	5,5	5,5
coût net	4,7	21,5	16,8
2 enfants :			
coût brut	4,4	27,0	22,6
prestations familiales	1,1	6,6	5,5
coût net	3,3	20,4	17,1
3 enfants :			
coût brut	4,1	27,0	22,9
prestations familiales	1,9	7,4	5,5
coût net	2,2	19,6	17,4

social). Il s'agit là d'un coût de prise en charge par la Sécurité Sociale et par les institutions analogues à la Sécurité Sociale qui pallient la carence ou l'insuffisance de la Sécurité Sociale elle-même.

En effet il existe nombre de lois complémentaires, de dispositions réglementaires et aussi d'actes privés (institutions privées, quêtes, associations charitables) qui viennent ajouter à ce que donne la Sécurité Sociale. Le chiffre que j'avance ici est une appréciation du total de la prise en charge par la société, soit sous la forme officielle de la Sécurité Sociale, soit sous d'autres formes. Et de la naissance à la mort; par conséquent ce chiffre tient compte de la vie moyenne de l'handicapé. J'avoue qu'avant de connaître ces chiffres j'aurais fixé cette vie moyenne aux alentours de 30 ans, par exemple — puisque la sur-mortalité des handicapés doit être forte aux âges faibles, aux âges adolescents, aux âges faibles, etc. Or, cette vie moyenne est de l'ordre de 50 ans. Le coût que je vais vous donner se réfère à cette vie moyenne de 50 ans de l'inadapté moyen. Pour

la vie entière, ce coût est de 500 000 francs de 1970, (soit 110 000 dollars environ), c'est-à-dire 10 000 francs par année de vie (*).

(*) Nous ne connaissons pas d'études sur le coût social des handicapés. Voici seulement quelques éléments d'appréciation.

ENFANTS.

Coûts extrêmes :

— Coût minimum : 600 francs par an.
Allocation aux mineurs handicapés (loi du 13 juillet 1971) : 50 francs par mois.
(Aide aux familles : surcoût par rapport à un enfant normal).

— Coût maximum : 13 000 francs par an.
Base de 60 francs par jour dans un internat spécialisé.
(60 francs par jour est un prix moyen, les prix de la journée pouvant aller jusqu'à 100 francs pour les handicapés profonds).

— moyenne de ces coûts extrêmes :

$$\frac{600 + 13000}{2} = 6\,800 \# 7\,000 \text{ francs.}$$

Il existe de nombreuses formules intermédiaires : intervention, aide sociale, externat, etc.

On peut considérer que les enfants sont pris en charge de 5 à 20 ans :

$$7\,000 \times 15 = 105\,000 \text{ francs.}$$

ADULTES.

Coûts extrêmes :

— Coût minimum :
Allocation aux handicapés adultes (loi du 13 juillet 1971) : 1 200 francs par an.
Cette allocation est donnée sans condition, indépendamment des ressources propres, alors que l'obligation alimentaire des parents joue pour l'aide sociale.

— Coût maximum : 20 000 francs par an.
12 900 francs pour handicapés bénéficiant de :
- allocation aide sociale ordinaire de revenu minimum;
- allocation aide sociale réservée aux grands infirmes travailleurs (9 275 francs au taux maximum);
- allocation donnée à tous les handicapés.

6 600 francs de frais de placement dans des centres d'aide aux travailleurs handicapés : 220 p. à 30 francs par jour.

— moyenne des coûts extrêmes :

$$\frac{1\,200 + 20\,000}{2} = 10\,600 \text{ francs par an.}$$

Compte tenu de la mortalité, on peut considérer que ces handicapés sont pris en charge de 20 à 50 ans :

$$10\,600 \times 30 = 318\,000 \text{ francs.}$$

COÛT TOTAL :

$$105\,000 + 318\,000 = 423\,000 \text{ francs}$$

Voilà Messieurs, quelques informations de base. Je serais très heureux de savoir si dans l'assistance on peut trouver des informations ou qui confirment ce que je viens de dire, ou qui, au contraire, y contredisent; et qui plus généralement nous apporteraient certaines des autres connaissances dont vous avez besoin pour éclairer votre action.

DISCUSSIONS

V. INGRAM : I just wanted to ask two technical questions : is the figure of 500 000 francs, the additional cost for a handicapped person without regard tot he cost to educate a normal child, is the 500 000 francs to be regarded as the additional cost ?

J. FOURASTIÉ : Oui, puisqu'il s'agit de ce qui est à la charge de la collectivité, de la société; donc c'est un supplément de ce que coûte à sa famille l'éducation d'un homme normal. Et, bien entendu, il faut ajouter que l'homme normal, lui, produit à partir de sa quinzième ou vingtième année, tandis que l'handicapé, s'il produit, ne produit évidemment que très peu.

Mais enfin, les 500 000 francs sont en plus de ce que coûte l'éducation d'un homme normal.

Par contre, le chiffre de 27 francs dont j'ai parlé, n'est pas un supplément : l'enfant normal coûte 4,50 francs et l'enfant inadapté coûte au total 27 et non 27 + 4,50 francs.

V. INGRAM : So, does the 500 000 francs take into account the fact that the person does not produce as the normal one ?

J. FOURASTIÉ : Non.

V. INGRAM : My second question was : is very any distinction made in this figure of 500 000 francs between the severely handicapped and the slightly handicapped children or is it an average figure ?

J. FOURASTIÉ : Il s'agit d'un chiffre moyen. Et c'est justement cela d'ailleurs qui donne à l'information un flou assez dramatique, parce que finalement, on ne sait pas très bien définir l'handicapé moyen.

Il s'agit de la prise en charge, par la société de l'individu moyen présentant à la naissance des anomalies physiques ou mentales. 500 000 francs soit environ 100 000 ou 110 000 dollars, représentent une somme énorme pour le niveau de vie français.

M. CEPEDE : Le coût des handicapés doit tenir compte des dépenses faites pour des enfants qui ne seront jamais des producteurs. D. Gosh a calculé qu'en 1945 — 22,5 % du produit national brut indien a été dépensé pour des enfants

qui n'ont pas atteint l'âge de 15 ans; à la même époque le chiffre correspondant pour le Royaume Uni était de 6,5 % — une différence de 16 % est énorme. Pour l'Institut de la Vie, plus particulièrement pour la Fondation McBride, il faudrait centrer nos recherches, — et beaucoup sont nécessaires — sur les handicaps résultant de la vie de la conception à la naissance — qui ont aussi des conséquences économiques très graves — Les premières expériences celles de John Boyd vers 1920 dans les sept principales villes d'Ecosse et Belfast intéressaient les enfants d'âge scolaire. Puis avec les travaux de Gyorgy et Anne Burgess l'accent a été mis sur le « Preschool child ». Notre problème est d'explorer ce qui affecte l'enfant de moins 9 mois environ à la naissance. Nous avons l'impression que beaucoup de choses que nous considérions comme « génétiquement héréditaires » proviennent des conditions du développement de l'embryon et du fœtus humain. Il ne s'agit pas seulement des agressions par les « drogues » mais aussi des carences alimentaires.

A. E. HELLEGERS : I would like to utter a word of caution about cost benefit analyses when one speaks of mental retardation and the handicapped. Ultimately all the things we find unacceptable can be cost accounted in such a way that if only a few people remained each would be a theoretical multi-millionaire. *I think the human misery factor is paramount.* Moreover the cost figures are often false. They include marginal care given by people, who, if they did not give the marginal care, might be unemployed and at the cost of the nation. Fractions of the special cost go to people who repay part of it to the state in income tax. The true cost can therefore once again not be calculated, because involved in it are hidden subsidies. Ultimately I believe cost benefit analyses could make one believe that no care is preferable to care. It can be counter-productive. Above all it loses the humanitarian function.

C. LEVINTHAL : I think I agree, in general, with the comment about the dangers of cost benefit analysis, but in spite of that I would like to ask since I am not quite sure that I know how multiplications goes, if you could translate the figures you gave us into a cost in terms of percentage of the Gross National Product.

J. FOURASTIÉ : Si vous le permettez, je dirai quelques mots sur les trois interventions. Avec M. Cépède, dans l'ensemble je souscris à ses vues, mais il a dit beaucoup de choses, et par conséquent il ne s'étonnera pas que tout de même sur certains points, je ne sois pas tout à fait d'accord avec lui. Il a évoqué le fait très important et très intéressant que l'homme moyen est certainement très inférieur en capacités intellectuelles et physiques, à l'homme optimum, si je puis dire. Le problème est énorme; on peut en rêver mais ce rêve est utile, et certainement très stimulant. On peut rêver de ce que serait une humanité dans

laquelle justement par la suppression de carences et de difficultés de tous ordres, l'homme moyen se rapprocherait beaucoup plus de l'homme optimum, et dans laquelle, par exemple, tous les ouvriers professionnels auraient l'habileté, l'ardeur, l'originalité, la vaillance des meilleurs ouvriers de France. Et de même, un monde dans lequel tous les professeurs d'Université auraient la valeur des plus grands noms de Harvard ou de la London School of Economics, pour citer des économistes. Ces exemples montrent justement combien la voie que vous avez envisagée ici depuis deux jours est une voie féconde et qui pourrait avoir des conséquences prodigieuses au point de vue économique même.

Cependant ma tendance serait plutôt de me rapprocher du second intervenant que du premier quand justement on veut juger de la place de l'économique dans l'affaire. Docteur Hellegers, vous avez senti tout ce qu'il y avait de prudence dans mon exposé et j'espère que cette attitude a répondu à vos préoccupations. Si vous jugez que je n'ai pas été assez prudent, je suis prêt à dire que c'est par maladresse d'expression, mais mon opinion est bien la même que la vôtre : l'aspect économique du sujet ne doit pas vraiment compter à côté de ses aspects humains. Ce comportement n'est pas seulement sentimental, affectif, il est plus qu'affectif, c'est vraiment de la vie même qu'il s'agit, de la quantité de vie, de la qualité de vie, de la possibilité même de vie. C'est dans cet esprit-là, je pense, que M. Marois m'a demandé de vous dire quelques mots. Tout de même si vous négligiez complètement l'aspect économique, manifestement il y aurait une grosse lacune dans vos travaux et nombreux seraient ceux qui vous dénonceraient comme des utopistes, comme des gens qui n'ont pas les pieds sur la terre et qui sont dans les nuages, qui évoquent des techniques n'existant pas encore pour prétendre au nom de ces techniques purement illusoires, avoir une action sur la réalité. Donc, je crois qu'il faut que vous en parliez, dans l'esprit que nous venons de définir pour dire que c'est second. Ma pensée sur ce sujet est très claire : c'est que votre cause, même si on l'exprime du point de vue économique pur, a un poids considérable puisque ce que je vous ai dit montre combien dès maintenant coûtent à la société, mais coûtent d'un point de vue purement sordide, coûtent en monnaie, coûtent en dollars, coûtent ces handicapés.

Par conséquent, cela permet de dire que maintenant il se dépense des sommes énormes et cependant ces sommes énormes sont tout-à-fait insuffisantes. Elles n'ont pour effet que d'apporter un petit élément d'aide, bien loin de correspondre à ce que dès aujourd'hui permettraient les techniques et à ce qui d'autre part serait désirable pour rétablir — je ne dis pas l'égalité — mais enfin un semblant d'égalité entre les handicapés et les autres.

Cela permet aussi de dire : que la prévention dont il est clair qu'elle pourrait coûter très cher, pourrait être pourtant rentable au sens le plus économique et le plus terre-à-terre du mot. La prévention coûtera sans doute moins cher

que ne coûte la situation actuelle avec son caractère dérisoire et tout-à-fait pénible.

Le troisième intervenant avait posé une question précise. Pour répondre, il faudrait faire un calcul, et dire : un Français sur 50 est handicapé. D'autre part, le handicapé moyen coûte par an environ 10 000 francs. Il faut comparer le coût total des handicapés au revenu national. Mais je n'ai pas le résultat présent à l'esprit.

W. McBRIDE : Humanitarian plus economic factors must be considered. Consider the child born with a meningomyelocela who will never be able to walk, will perhaps never be able to control his bladder.

K. H. DEGENHARDT : The calculation for incidences and costs of handicapped children is quite the same in German Federal Republic as in France. In spite of the high costs, it is a matter of humanity to increase all efforts taking care of the handicapped people.

The main task of teratology may be to prevent congenital malformations.

How low the costs could be in prevention may be explained by the following example :

Professor Vogel (*) in Heidelberg calculated the rates of newborn children with Down syndrome in the years 1950 and 1964 within thy German Federal Republic. He stated a 30 % higher incidence in 1950. This may be due to the decrease of pregnancies in higher age groups above 35 years and an increase of pregnancies beneath 29 years.

M. BJELIC : Both prevention and rehabilitation services are necessary, since a certain number of " new " handicapped persons appear every year. It is difficult to measure the economic impact of millions of handicapped persons already employed who do not appear in any statistical counts after using rehabilitation services over a few years.

J. FOURASTIÉ : Je crois que toutes les observations faites sont très pertinentes. Ce qui est essentiel c'est que nous soyons en mesure d'affirmer que dans beaucoups de cas, la prévention coûtera moins cher que la cure — et l'exemple donné par notre collègue allemand est très caractéristique. Cette notion est d'une importance considérable puisque à la fois la prévention est infiniment plus efficace et que, au moins dans certains cas, elle peut coûter moins cher. Même si elle coûtait plus cher, l'effort vaudrait la peine.

(*) F. VOGEL, Wie stark ist die theoretische Häufigkeit von Trisomie-Syndromen, in *Zoologische Beiträge N.F. Bot.*, **13**, 2/3, 451 (1967).

CONCLUSIONS

J. EBERT : Inspired by Dr McBride, the Institut de la Vie has taken its first steps in a direction new to it. In these three days, we have been grouping, all of us, not for immediate solutions of problems that are as old as mankind itself, nor for an immediate identification of the role of the Institut de la Vie, but towards a first preliminary understanding of the steps that we, as a group, might be able to take, leading towards the ultimate solutions of these compelling problems.

Each of us comes away from any Meeting with his, or her own impressions of it, and perhaps the range of impressions of any meeting are almost as varied as the number of participants therein.

My own impressions of the meeting are in part summed up by my adjective *groping*, a word which has characterized our halting movements, usually forward (two steps forward and one back). None the less, this afternoon I leave with certain very clear impressions.

First, in the contributions of the Boué's, we have heard presented a potentially highly significant set of observations which, when verified in the months and the years to come, call very strongly for a reevaluation of the clinical approach to the maintenance of pregnancies which are endangered by one reason or another, especially early in the term. Whether these observations will be sustained, remains to be seen but as this point in time, I have a clear impression of their importance (a clear impression, that is for a person who is not clinically trained).

A second impression which I gained here, or that has here been reenforced is a clearer feeling and understanding for, and appreciation of the power of the epidemiological approach, which as presented by Dr Miller has awakened me to possibilities which I had not fully understood before.

I think, thirdly, I saw to my dismay that despite eleven or twelve years of major international conferences on the subject, involving groups as diverse as this one, that the gap between the clinical and the basic scientists remains nearly as large as it was at the first International Congress on Congenital Malformations in 1960. Perhaps this is my most negative impression of meeting. It is an unfortunate one but to me at least it appears to be true.

It is very difficult to come to grips with the question of how one crosses bridges. This gap I think is not merely a "generation gap" but a real gap in understanding of the approaches of the several fields here represented. I think I am especially sensitive on this point, for it has seemed to me that in other areas, in the area of tumorigenesis for example, the gap between the clinical and the basic research laboratories has narrowed rather than widened in recent years. In the study of congenital defects, however, the problem still remains to a very large extent. In my own view, the common meeting ground is genetics, a field in which molecular and cellular biology certainly converge with the clinical aspects of the subject in a very effective way, as Professor Degenhardt has emphasized repeatedly in the course of these days. Perhaps one of our problems at this meeting was the lack of a representative of the field of somatic cell genetics, an area in which the cellular and molecular approach to genetics begins to interact with human genetics and cytogenetics.

These have been merely my own impressions; they are not intended in any way as a summary. I should like next to call upon someone from whom we haven't heard in the course of the meeting. He represents an Organization which since 1958 or 1959 when it first entered the subject, has been groping for solutions to these problems, trying new approaches, keeping some, abandoning others and trying new ones. Mr Salisbury, would you like to give us your impressions of the Conference and some of your thoughts during these three days.

A. SALISBURY (Summary) : I described the program of the National Foundation in terms of :

1. Medical service
2. Research
3. Professional education
4. Public health education and community services.

I suggested that the Institut de la Vie considered the following for program activities :

1. Monitoring and surveillance of the incidence of birth defects
2. Providing a central ressource for information regarding treatment and research in birth defects
3. Financing foreign training for researchers and clinicians
4. Study of the legal, moral and ethical aspects of genetic services.

J. EBERT : Thank you very much. Am I correct in that your only international activity is in the sponsoring of the international conferences ?

A. SALISBURY : No, that is not quite correct. We do allocate funds usually in small and non repetitive amounts for various activities, for exemple, the

European Teratology Society has been recently given funds for their conferences of which we did not participate directly; other international activities are funded on prior purposes, I should say.

J. EBERT : What is your feeling about the level of similar activities throughout other parts of the world ? Are there other countries that are ahead of the United States in doing better what you have been doing ? Are there other models that we can look to in any of these fields ?

A. SALISBURY : Well, I think that the number of the voluntary Help Agencies, there probably are very few like examples in any place in the world. I know there are voluntary Help Agencies in New-Zealand, and in Australia which Dr. McBride could describe and I wouldn't confuse it by doing it, but the voluntary Help Agency movement is almost peculiar to the United States as I understand is heart, cancer and polio in the old days. American are incorrigibly independant when it comes to their health services; everybody likes to have his own disease foundation so we have a number of them. I don't think any other country has quite that experience.

J. EBERT : Thank you very much. I wonder now, whether Dr Miller, would like to give us now your impressions of the last several days ?

J. MILLER : I was impressed, on the first morning to see how many people were busy copying down the statistics which Dr McBride put on the screen. I guess when you are familiar with such statistics and the problems involved with their collection, you become somewhat blase. To me these statistics were old hat and while they provided a good introduction to our symposium they were not really that dramatic. I should like to have known, for example, about annual, seasonal, and other variations. However, it is obvious that such raw statistics as presented by Dr. McBride still have a great fascination. Most people still cannot believe the magnitude of the problem with which we must contend. But it should be emphasized repeatedly that we really do not know for most countries the frequency with which these events occur. In this day and age when we are discussing rather elegant and sophisticated biological systems for the study of etiology and developmental mechanisms, the apparently very simple job of counting babies and anomalies and recording this information in meaningful form seems to be an impossibly difficult task in most places, including the so called developed countries.

I am concerned primarly about two issues : first of all how do we translate into practical programs many of the techniques that are now available to us for prevention ? For example, I think too frequently couples after the birth of

their first malformed child are told that the causes of congenital anomalies are mystery which no one can understood, that is no real risk of having another defective child and they should certainly try again. However, all this information is given without any attempt at an investigation and is only when this couple produces a second or even third malformed child that they are referred to some expert who will look into the situation in greater depth. Arthur Salisbury referred to several programs sponsored by the National Foundation which involved the application of procedures that are available now. My concern is the population receiving these services even in the United States represents only one small part of all those who require them.

A second matter which concerns me is despite the warning of the Thalidomide episode (and it is ten years old now) most communities are unable to measure the presence of a new teratogen in the environment and have no procedures established for following up in the detection of a specific teratogen even if its presence was suspected. I don't believe that the studies which I advocated in my presentation are in any way complete in themselves. However, I do believe that the collection of reliable statistics on the frequency of occurrence of congenital anomalies provides a base for a variety of additional studies. Without these baseline statistics I think constructive monitoring projects will just not materialize for years to come.

Finally, I think we need to use every tool we have available to us and I think that the presentation of Professor Nishimura is a good example of this point. He and his Japanese colleagues have used embryos and fetuses to study many of the problems with which we are concerned here. Yet, in most parts of the world this important biologic material is wasted.

These are my general impressions. I believe that these are issues which we can deal with now. At the same time secondary and backup programs we should encourage more of the basic research which we have heard about this meeting and which we anticipate will provide in turn more tools which can then be applied in preventive programs. Thank you.

J. EBERT : Thank you very much. Monsieur Marois and the Institut do not wish to attempt to formulate a specific programme of action at this meeting or even in the immediate days to come. Over the next few months, however, they hope, with the assistance of many of you, by mail and in other ways, to begin developing a plan of action. It would be useful, then, to have at this time expressions of opinion from any of you on any specific points that have not yet been brought to the fore. Your critisisms, or other comments are now welcome.

V. INGRAM : As regards general comments on the conference, I would just like to say that I for one have learned a great deal, many things that I did not know

before and I found it in some respects, let us say, a shattering experience, particularly from a biological point of view, the problem which you mentioned Jim, the observations rather that only one out of two fertilised eggs goes on to term and all that it implies in terms of basic biology and also in terms of the question whether it is wise to push on with the life of such embryos.

But I really wanted to talk about a rather different topic : I was very distressed to hear no discussion at all of one of the most serious, most frequent instance of congenital malformations, namely Sickle Cell anaemia which is certainly a congenital malformation in many a sense of the word about which we know a great deal in terms of molecular biology but about which at this point very little has been done or until recently very little has been done from the point of view of the population. Now I know that, for example, the National Foundation is involved in organizing a conference on Sickle Cell Anaemia in New York just recently I believe, and the whole question of the occurrence, the detection, the treatment of Sickle Cell Aneamia has become a very hot issue, both politically and from many other points of view in the States in recent months. The frequency, of course is very high indeed in the United States alone, of the order of a thousand homozygotes are born every year and they are certainly very seriously ill, if not exactly lethal necessarily. The incidence in Africa and other parts of the World is even higher so that enormous numbers are involved.

The point I want to raise is this and this is where I would have hoped we might have discussed or may be discussed it at some future occasion : the techniques for screening a large population for the occurrence of Sickle Cell heterozygocity either exists or is just round the corner, the techniques to do it sufficiently, efficiently, clearly and relatively cheaply. And there are now, in fact, a number of independent Agencies and independent groups, some of whom are involved in screening programmes of this kind on a fairly large scale in the States. I have the feeling that the efforts are not coordinated, and maybe I am wrong about that, maybe Dr. Salisbury knows more about this than I do. The whole question of whether one should, whether one is justified in putting on such a large screening survey, justified from a medical point of view, justified from an economic point of view, this is the whole question about which. I find myself totally confused and I would wonder whether other people have clearer ideas about it. It has become a very urgent problem in the States, in the sense, as I said, that a number of groups are pressing for this; it is not clear what you tell a person who is detected to be a heterozygote and they are in the order of 1 500 000 of these in the States alone, what you can offer in terms of genetic counselling and whether they accept genetic counselling and things like that. But it is my purpose today to raise the issue; it is one which a group — and you mentioned the Institut de la Vie — should do properly, I think, to concern

itself with, because it is an immediate problem, not one that is going to wait for a couple of years.

This is a problem right now. It is a problem also because technological advances are going to be very rapid in that area, because there has been an infusion of money into work in this area : the biochemists are talking about a possible therapy for the disease. This is a long way off but they are talking about it and this is being discussed all of which would influence on's attitude towards the desirability or undesirability of conducting or setting up surveys on a large scale in Sickle Cell aneamia or Thalassaemia which is also a very frequent disease. And I would like to raise this very important problem to people's attention, to the people who are better qualified than I. I thought about this and whether this is a proper subject for the Institut de la Vie.

S. LARSSON : I would like to take the opportunity before we close this Session to thank you very much for the organization of this meeting, one of its important items was to bring scientists of different disciplines together. It is important to bring people not only from different disciplines but also from different countries together. In the European Teratology Society we have had difficulties to bring researchers from East and West together. But we have had valuable economical help from the National Foundation, March of Dimes to facilitate such a problem. And I would like to stress the importance for an organization as the Institut de la Vie to contribute in contact service either by conferences or by post-doctorate fellowships.

Another problem concerning contacts and information is the rapid and overwhelming flow of articles and information. We must do all efforts to retrieve the literature within a relatively short time period and have the pertinent articles on congenital malformations collected in a database. I would be happy if the new organization and Foundation could go into such activities. Thank you.

J. EBERT : Thank you very much. Is there any further discussion of Dr. Ingram's statement ?

A.E. HELLEGERS : I would simply express the hope that the Institut de la Vie would not duplicate the efforts of other Institutes or Foundations. It would not just be duplication of effort but you could not manage it financially. I would therefore hope that you would specialize in brains and astuteness in an area not covered by other agencies.

J. EBERT : Thank you, Dr. Hellegers. Dr. Goodwin, please.

B. GOODWIN : I hesitate to say very much in this area because I feel I am an outsider but I have certainly learned a great deal during the past three days. What impresses me about what has been discussed is that if one could tabulate in some way priorities with respect to the major factors which are concerned with congenital abnormalities, then possibly some kind of publication or statement could be released by the Institut de la Vie as part of the programme whereby this organization could attempt to act as a lobby in the domain which is rather sensitive between research, medical investigation and the initiation of programmes which are partly political and partly scientific. I have in mind, for example, that if we tabulate the possible sources of congenital disturbance such as malnutrition, infection, environment, drugs, aged parents, etc., then the goals for amelioration of conditions would include attempts to limit population growth, legislation to allow therapeutic abortion, the surveillance and screening methods that Dr. Miller has mentioned, legislation for control of drugs and potential teratogenic agents. In all these areas I would have thought that the Institut de la Vie could act as an intermediary between the authorities of scientists and doctors and the political domain which is clearly involved in initiating programmes which can realize the objectives of this conference.

C. SWINYARD : I believe that we should avoid picking out a particular birth defect for particular emphasis because it is too difficult to establish priority. For example my particular birth defect of special interest is myelomeningocele. Nearly 10 000 of these children are born with this defect annually in the U.S.A. We have four hundred active patients in our clinic. I believe it would be better to consider general programmes which would increase interest and competence of professional personnel upon broad questions on maldeveloped, education and information exchange.

J. EBERT : Dr. Ingram do you wish to respond to that point specifically ?

V. INGRAM : I would agree with you. I would not wish to say that " You know my defect is worth more than your defect ", or anything like that. But there are two important issues which I don't think you responded to. One is people are, in fact, going to do something about it but probably and many of them, in an ill-advised and haphazard way, as far as I can see them, but may be I am wrong, this is not being properly coordinated and has not been thought out, at least this was the impression which I have very strongly and other people too at the New York Conference the week before last on Sickle Cell anaemia. So in other words, it is an immediate problem where something is going to happen and we want to make sure that something useful is going to happen.

The second thing is that technical advances in this particular area are very rapid and they are real both in terms of detection and at least the hope is there for some kind of therapy, so I think these are two additional factors. I don't know : I raised the question. I meant it as a question. I don't know whether it is appropriate for the Institut de la Vie to consider this but I would have wished somebody would.

I mean I would agree that it was very good that the National Foundation organized this Conference in New York which was the first of its kind. It was very successful in terms of numbers. My feeling and that of the people round about me sitting in the Hall, was that it was very unsuccessful in terms of formulating any goals, policies or even agreement on facts. So I would from the point of view of the objective of the Conference, I think, not the biology or biochemistry — that was O.K. — but that was not the issue, but in terms of what to do about this problem in the population, which was one of the objectives, from that point of view, I think that it was a very unsuccessful conference.

J. EBERT : Dr. Winick and then Dr. Moscona, please.

M. WINICK : It seems to me, you know, that somebody raised an issue just a moment ago that we might want to elaborate on. And that is the fact that a lot of the situations that we are talking about now, those situations in which some abnormality in development — call it a birth defect if you want to — Sickle Cell Aneamia, the effects of malnutrition on the brain, or on any of such things, interest has been generated in this because of political events and we have have to face that. I mean the interest that has come in Sickle Cell Anaemia has come as a result of political events. The interest in malnutrition on the brain for which there has been great evidence for the last fifteen years, has come as a result of a CBS Television programme, at a White House Conference. Now, once we accept that political events are generating these kinds of interest in these things, maybe that we ought to take a look at the possibility of an organization such as this at least getting itself into a position where it may be able to interpret some of the scientifically relevant data to the political policy-makers who are influencing these events. And I think this may be a reasonable role for an organization of this type, a thing it should think about in terms of what it can do which is unique. I really don't know of any organization which has taken this as an active role to attempt to make this kind of a bridge and I think it is an exceedingly important bridge to make because I think that is the only way something is going to get done.

A.A. MOSCONA : I would like to make a somewhat more specific proposal

which might serve to bridge some of the ideas generated during this discussion. It is based upon some of the impressions that occurred to me during this meeting.

First of all, we have all learned a lot during this meeting, specifically because it brought together people from different disciplines; although we occasionally reacted with a smile of disinterest to eacher other's comments, on second thought we appreciate what we have heard, and recognize the importance of communication across disciplines.

Next, it should not be overlooked that the National Foundation with the background of its tremendous experience is putting so much effort into education; this is important because, as we have clearly found out here, there is a definite need for cross-education between scientists from different disciplines interested in congenital malformations.

I therefore suggest that one of the tasks that might well befit, at this stage, the Institut de la Vie could be a series of small interdisciplinary workshops directed specifically towards discussion of problems of congenital malformations in the context of medical-biological and social issues. By bringing together small groups of scientists from different disciplines, such workshops would help to focus attention to various aspects of this problem and its diverse ramifications. I agree with the comments that there are, in general, too many meetings; however, this is a special situation and although the problem of congenital malformations is of such importance it has not received adequate attention of the kind to which multi-disciplinary workshops can lead.

We have learned a lot from each other about each other's work and about the topic as a whole during the informal discussions at our lunch-breaks. Taking a cue from this, it would seem that informal workshops on various aspects of congenital malformations could be very useful and their organization might represent an important function of the Institut de la Vie.

J. EBERT : Dr. Ingalls, would you like to comment ?

T. INGALLS : Well, I would just like to underscore the last speaker's comment that the informality of this gathering is a great asset. Also, I should think we would do well to avoid bringing a local American political issue into our deliberations here. Nor do I think we are ready to declare for any particular course of action against any real of hypothetical teratogens. Once a grave danger is exposed, people of all races and nations can be counted upon to unite in their opposition to it. This is evidenced, for example, by progress that has been made all over the world in rubella control and thalidomide removal. Perhaps it is worthwhile to review very briefly, the steps which led to their indictment and to emphasize that the first step is a clinical recognition of the congenital defect in

a newborn baby or young child. Modern understanding of rubella embryopathy came out of Australia when the disease was epidemic during the second World War. When the British Commonwealth mobilized in 1939, many young men, and more important, their young wives and sweethearts, had never had German measles in their lives. And so, conditions were ripe not only for an epidemic of rubella among children, but also among young adults, many of whom were women in early pregnancy.

As you all well know, the modern understanding of thalidomide risks also came out of Australia and it was Dr. McBride's observations at the Women's Hospital in Sydney that led to the recognition of the extraordinary event that we are marking by our very presence here.

For those of you who don't know this, I will say that thalidomide (Contergan as it was called in Germany, Distaval in the British Commonwealth) was, on the surface of things, a very acceptable hypnotic for a good night's rest, seemed to have no hangover like barbiturates and seemed to have little risk of overdosage or of being used by a suicidally minded person. What more natural than that it should be tried out eventually for treatment of morning sickness at a Lying — In Hospital in Sydney, Australia, for example. Thus, the first chapter in the thalidomide episode seemed to be constructed like a fictitious plot. Dr. McBride's role, like Gregg's, was as first observer, to recognize the enormity of the clinical defect in relation to a postulated cause — thalidomide ingestion.

Secondly, he tried to assemble the evidence he could gather to support his opinions and to this purpose prepared a paper for one of Britain's most prestigious journals. Back it came, rejected, however, because the argument was just unthinkable. The macabre consequences of a few pills just couldn't be that bad. Dr. McBride finally prevailed upon the Editor to let him at least publish a letter. I keep a photostat of that letter in my correspondence file. I treasure it, because it is as important a short letter to an editor as was ever published — of exactly 100 words in the December 16, 1961 issue of the Lancet, to say that the writer had seen several cases of " multiple severe abnormalities in babies of women who were given thalidomide during pregnancy... Have any of your readers seen similar abnormalities in babies of women who have taken this drug during pregnancy ? " asks the writer. And this was without the assistance of a computer, simply on the basis of observation. Probably I have spoken too long. I mainly wanted to say that I think it would be presumptuous of us to come all the way to Paris as guests to try to advise our hosts about a particular problem of our own. Also I think you have two wise consultants in the back row here — Dr. Warkany and Dr. McBride. The reason why I mentioned Dr. Warkany right now is because, Dr. Ebert, you have brought up the gap existing between the basic science

workers, the clinicians and the public health workers. We all agree in our more convivial moments there should be no gap; but is this really so ? A little while ago I overheard Dr. Warkany talking informally with Dr. McBride and someone else; Dr. Warkany was making a profoundly simple comment that has to do with this very gap, i.e. the gap is inherent in our professional training, our expertise and our activities. The physicion's first responsibility is to his patients, to deliver their babies, to relieve their symptoms and, as and when possible, to cure them or better yet to spare them illness or congenital defects, the costs of which are incalculable. In contrast to the medical practitioner the basic scientist is working largely in his laboratory — with his enzymes, bacterial cultures, viral systems, chromosomes, microscopes and colorimetric analyses, etc. The public health servant is preparing charts of infections, of live births, or crippling conditions. He is using the computer and tests for statistical significance more freely than either the clinician or the molecular biologist. Why should we pretend that we each understand the other's milieu and that the real truth and the real expertise goes with the initials that we put after our names. We need each other. We need to communicate with each other. And we don't have always to agree with each other.

A final word, and I'm sure I speak for my colleagues. We have all enjoyed your hospitality, it has been a particularly interesting conference, more than interesting to me. I see these things clarified — the difference between cytogenetics and teratology; the distinction between phenotype and genotype and relevance of our discussions to basic science, clinical medicine, public health; yes, and to ecology and natural history. We have accomplished a lot and I would like to make a suggestion that if our proceedings are published, they should include a reproduction of that little letter to the Lancet, because that is a large part of what we have been talking about. That letter would make a good preface.

J. EBERT : Thank you very much. Are there further comments ? Dr. McBride, would you like to comment at this time ?

W. MCBRIDE : The main function of this meeting has been to bring to the attention of scientists, politicians and the public the importance and the extent of the problem of physical abnormalities, mental retardation and cerebral palsy.

I agree with Dr. Winick. I think one of the most important things which have come out of this when Jim Miller described my statistics as « old hat », and this is certainly true that did not offend me at all, but the thing that I think we should find offensive is that these old hat statistics have been occurring in the year 1971, when we think that we are in a very modern era.

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THALIDOMIDE AND CONGENITAL ABNORMALITIES

SIR, — Congenital abnormalities are present in approximately 1.5 % of babies. In recent months I have observed that the incidence of multiple severe abnormalities in babies delivered of women who were given the drug thalidomide ('Distaval') during pregnancy, as an antiemetic or as a sedative, to be almost 20 %.

These abnormalities are present in structures developed from mesenchyme — i.e., the bones and musculature of the gut. Bony development seems to be affected in a very striking manner, resulting in polydactyly, syndactyly, and failure of development of long bones (abnormally short femora and radii).

Have any of your readers seen similar abnormalities in babies delivered of women who have taken this drug during pregnancy?

Hurstville, New South Wales. W. G. MCBRIDE.

*** In our issue of Dec. 2 we included a statement from the Distillers Company (Biochemicals) Ltd. referring to "reports from two overseas sources possibly associating thalidomide ('Distaval') with harmful effects on the fœtus in early pregnancy". Pending further investigation, the company decided to withdraw from the market all its preparations containing thalidomide. — ED.L.

Reproduction de la publication princeps sur l'action tératogène de la thalidomide.

But yet our retarded children, physically abnormal children, now their incidence is no lower, perhaps it is even higher than it was thirty or forty years ago. We have made all sorts of advances in medicine in the last thirty years; Highlighted by the control of Malaria the advent of chemiotherapy, antibiotics, but yet we have done nothing about reducing the incidence of physical and mental abnormalities.

I think that — Jim Miller said that some people wrote down these statistics because they were new to them — if this meeting has done nothing else but get across the enormity of this problem that you have got 5 per cent of children born in this atomic era with very severe abnormalities, either mental or physical and then in addition, another 7 per cent with perhaps milder degrees but enough to affect their lives, making a total of 12 per cent, well, it is something which I think we shouldn't be very proud of. I thank you.

J. EBERT : Thank you very much. Dr. Warkany, would you like to comment?

J. WARKANY : Well, there is just a word I want to say that if a wise man like Dr. McBride is questioned, if his statements are questioned, he is not offended, that is very important and I am referring to yesterday's discussion.

Secondly, I think I don't know what you are planning to do here, but in discussions with Dr. McBride, I found out that he has very definite ideas about the Foundation in Australia and I must say I agree with him completely. It is a very strange phenomeron that has not been mentioned here, that congenital malformations which develop under the care of the obstetricians are not considered by obstetricians very much; it is pediatricians who, for decades, have worked in the field of congenital malformations and more recently the internists followed. As far as I can see, obstetricians are still not aware of the problem; they are not aware that it is their job to prevent congenital malformations or to do something about it. Dr. McBride is one of the few who has seen the problem, who, I hope, will direct the young generation of obstetricians first in Australia and may be later in other countries, to this very important field and I think he doesn't worry about the use of the funds that will go to the Foundation that I hope he will head.

J. EBERT : Thank you very much. I have no desire to terminate the discussion if there are major points still to be made but unless there are questions of some urgency, I think the time has come for more of that informal conversation that I known all of us value at such conferences. Before closing, I should add that Monsieur Marois and the Institut de la Vie would like to have expressions of your views concerning publication. You might to comment to Mr. Marois now, at the close of the meeting, or perhaps by letter, whether you would favour publication of a more or less complete record of the meeting or whether you might prefer something in the form of a brief meeting Report to be sent to *Nature* or *Science*. I known that he will value having expressions of your views.

I realize that many of us will be together this evening socially, but while we are still here assembled, we should take this opportunity, to thank Monsieur Forestier, our host, and this remarkably fine group of most charming ladies and gentlemen who have been with us all through the meeting, our interpreters and « collectors » of all of this valuable information. Perhaps they can leave off translating for a moment and come out and take a bow, along with these other lovely young ladies who have been with us (General applause), especially Mrs David who has been endearingly spoken of as our « den-mother » — Oh ! here she is now, our « den-mother », Mrs David, our sincere thanks. (Applause and laughter) Thanks to each and everyone of you.

M. MAROIS : Je vous remercie, Monsieur le Professeur Ebert, d'avoir avec une

telle délicatesse adressé les remerciements du congrès à tous ceux qui avec tant de bonne grâce et d'efficacité ont contribué à sa réussite. A mon tour, au nom de l'Institut de la Vie, au nom de son Conseil d'Administration et en mon nom personnel, j'exprime notre très profonde gratitude au Dr. McBride, aux rapporteurs, à tous les participants pour leur science, leur liberté d'esprit et leur souci du bien commun. Je dis enfin notre reconnaissance à tous ceux qui ont rendu matériellement possible cette rencontre, à Jean Chenevier, à Charles Mérieux, et tout particulièrement à nos hôtes : Denis Forestier et la Mutuelle Générale de l'Education Nationale; avec un sens exemplaire de la solidarité entre les hommes — ce qui ne m'étonne pas, les connaissant —, ils ont tenu à nous recevoir; et nous nous plaisons tous à reconnaître la discrétion, l'élégance, la générosité de leur accueil.

Je voudrais enfin terminer sur quelques paroles d'espoir. Comme vous l'avez dit, Monsieur le Professeur Ebert, nous faisons les premiers pas sur une route difficile. Les problèmes que nous rencontrons, confrontent l'humanité depuis qu'elle est l'humanité, et il est bien clair qu'en une réunion, nous ne pouvons pas tout résoudre. Vour l'avez senti : il ne s'agissait que d'un premier pas. Grâce à vous tous, je suis profondément convaincu qu'il y aura un second pas. Merci.

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