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

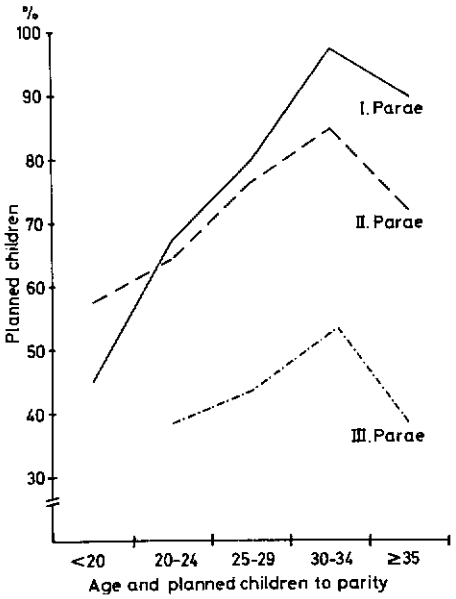

TABLE 3

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.