Meetings

Hormones in Development

Hormone action in development up to now has been analyzed in three ways: (i) The "fundamentalist approach" aims to demonstrate that hormones influence and participate in the control of differentiation of various target structures. The demonstration relies essentially on procedures that either remove the source of hormone secretion or that administer excess hormone during fetal life; the effects of such treatment are then observed. (ii) The "analytical approach" focuses on critical periods during embryogenesis in an attempt to identify the specific developmental processes that are subject to hormonal interference. (iii) The third approach attempts to interpret hormone action in terms of ultimate cellular mechanisms and to fit the effect of hormones on target structures with the "central doctrine of experimental biology" according to which differentiation reflects the emergence of selective protein synthesis, an event which is presumably preceded by the differential repression or derepression of genes that code for the different proteins.

Evidence for direct influence and interaction of hormones on a variety of developing target tissues was explored, at an international conference on "Hormones in Development," held at Nottingham University, Nottingham, England, 9–12 September 1968.

Florence Moog (Washington University) reviewed the role of corticoids on the functional differentiation of the intestinal epithelium. Moog stated that "corticoids apparently act in ontogeny by directing differentiation into pathways that become partly independent of the inciting agent. Thus, in the mouse or rat, cortical hormones induce a 20- to 50-fold increase in alkaline phosphatase activity during the third postnatal week, but in the adrenalectomized adult phosphatase activity, though reduced below the normal level, remains far above the level of infancy. . . ."

Jacques Roffi (University of Paris) reported results that indicate that corticosteroids regulate the activity of the enzyme which methylates norepinephrine to epinephrine in the fetal adrenal. He deprived rat and rabbit fetuses of their hypophyses by decapitation before adrenocortical functions began. At term their adrenals contained only one fourth of the normal content of norepinephrine. Administration of adrenocortico-stimulating hormone (ACTH) or cortisol to the fetuses completely prevented this effect. Roffi speculates that the intimate association of adrenocortical and adrenomedullary tissue during the early development of the rat is necessary to provide the medulla of the adrenal with a high concentration of corticoids required to stimulate the methylation of norepinephrine to epinephrine.

Organ culture was used to clarify the relation of fetal sex hormones to the normal development of the reproductive tract by Price, Ortiz, and Zaaijer (Universities of Chicago, Puerto Rico, and Leiden, respectively). Their results indicate that (i) fetal testis is secreting androgen at 22 days of gestation, before it is recognizable as a testis, and that the Wolffian ducts are completely dependent on testicular androgen by 26 to 27 days (ambisexual stage); (ii) fetal testicular androgen does not inhibit female Mullerian ducts but stimulates the retention of the Wolffian ducts; (iii) the Mullerian duct is hormone-independent through early critical developmental stages, particularly in the oviduct segment and; (iv) with androgenic tests, metabolic activity in the ovary is not detected until 41 to 46 days, although marked activity is detected later.

S. E. Levina (Academy of Science of the U.S.S.R., Moscow) reported that the sexual differentiation of the hypothalamus as it relates to its capacity to stimulate the pituitary to secrete the follicle-stimulating hormone (FSH) seems to depend, in the human fetus which provided the material for organ culture, on the presence in the culture of either male or female pineal gland. Human female pineal gland added to the culture increased secretion of FSH; male pineal gland inhibited it.

The well-documented direct response to thyroxine of amphibian metamorphosizing tissue was the subject of considerable discussion, for the obvious reason that this tissue presents a target particularly suitable for analysis of the mechanism of hormone action.

Albert Derby (City College of New York) demonstrated that the pituitary (pars anterior) of the tadpole releases a factor that interferes with the action of thyroxine on tail disk resorption in vitro. His results are interpreted to indicate that an antagonism exists, with respect to tail resorption, between a pituitary growth factor (possibly a prolactin-like, or a growth hormone–like molecule) and thyroid hormone. This interaction takes place peripherally through direct interactions at the site of the target cell.

Ki-Han Kim (Purdue University) reported that during natural or thyroxine-induced metamorphosis, the pancreas loses from 70 to 80 percent of its original weight as a result of actual necrosis, not dehydration. The developmental processes underlying the regression are under thyroxine control and occur in a definite chronological order. An increase in acid phosphatase activity accompanying the regression results from the activation of a preexisting enzyme and not de novo protein synthesis.

The second most extensively investigated structure in developmental endocrinology is probably the maturing brain and the effect of thyroid hormone on neurogenesis.

Eayrs (University of Birmingham, England) reviewed his theory according to which the defective performance presented by hypothyroid rats, when confronted with conditioned response or standard neurological tests, is caused by a hypoplasia of the developing neuropile consequent upon thyroid deprivation in the young neonatal mammal.

Balazs (Medical Research Council, England) in collaboration with Eayrs reported that in brains of hypothyroid

rats the activity of glutamate decarboxylase, an enzyme relatively concentrated in the synaptosomal fraction of brain homogenates, as well as glutamate dehydrogenase localized exclusively in mitochondria of nerve perikarya is considerably depressed compared with controls.

I. Pesetsky (Albert Einstein College of Medicine), using electron-microscopic and histochemical methods, has found marked changes in a number of enzymes such as glucose-6-phosphate dehydrogenase, NADPH diaphorase, and thiamine pyrophosphatase (TPPase) and changes in the endoplasmic reticulum in brain cells of tadpoles that were either treated with exogenous thyroid hormone or thyroidectomized. These alterations were located in the ependymal glial cells, raising the whole question of whether the glial elements rather than the neurons are the thyroid dependent targets in the tadpole brain.

In the absence of thyroid hormone, the delay in the disappearance of the external granular layer of the cerebellar cortex leads to permanent changes in the Purkinje cells in cerebella of hypothyroid rats. It was suggested that a failure in timing—the delay in disappearance of one cell layer—has lasting effects on the differentiation on another cell population, that is, the Purkinje cell (J. Legrand, University of Montpellier, France).

Furthermore, in the absence of the thyroid hormone the cells of the external granular layer of the cerebellum continue to proliferate beyond the time at which in controls such proliferation has already ceased. However, excess thyroid hormone administered from birth on reduces the duration of the proliferative phase (M. Hamburgh, City College of New York and Albert Einstein College of Medicine). Hamburgh suggested that one way by which normal levels of thyroid hormone may influence central nervous system development is by turning off the proliferative phases in time so that the differentiative phase (RNA synthesis) can begin.

This interpretation found some support in that thyroxine administered to chicken eggs at 9 days of age lowered brain DNA, whereas thiouracil treatment increased brain DNA by about 10 percent at hatching. By day 21, the DNA was normal in both groups (S. Zamenhof, University of California, Los Angeles). Zamenhof suggested that thyroxine may affect cells, during their proliferative phase.

The mutual exclusiveness between proliferation and differentiation, suggested by these reports, is in line with basic assumptions of developmental biology and holds for a large number of cases examined. That the timing of these processes may be under hormonal control is less well known. The paper read by Yale Topper and A. E. Voytovich (National Institutes of Health) was relevant to this assumption. They reported that studies on the developing mammary gland led them to postulate that the formation of a clone of daughter cells competent to develop specialized synthetic activity is itself under hormonal control. In developing mammary glands, insulin initiates DNA synthesis in explants of the gland of the immature animal. Sometime between the onset of such synthesis and the end of mitosis, both insulin and hydrocortisone are necessary for the formation of competent daughter cells which, in turn, become producers of casein after exposure to prolactin and insulin.

The assumption that ultimately hormones stimulate synthesis of specific proteins either at the level of transcription or at the level of translation was defended by Claude Villee (Harvard Medical School). He reported that the injection of testosterone increases the biosynthesis of RNA in seminal vesicles and prostate, as measured by the incorporation of ^{3}H-cytidine. RNA extracted from the seminal vesicles but not from other tissue 12 hours after the injection of testosterone, when instilled into seminal vesicles of control 3-week-old rats, stimulated growth and protein synthesis. The stimulatory effect was eliminated if the RNA preparation was treated with ribonuclease or was heated 15 minutes at 100°C.

In further experiments, RNA preparations were injected into one lobe and saline into the contralateral lobe of rats castrated when 5 weeks old. RNA from the seminal vesicles of castrated males injected with testosterone was as effective as RNA from the seminal vesicles of intact adult male rats. In contrast, RNA from the liver of adult female rats was ineffective whether or not the animal had been injected with testosterone.

Villee speculated that testosterone enters the nucleus of the target organ and stimulates the production of a particular species of RNA which can then be extracted, partially purified, and reinstilled into the seminal vesicles of control rats and produce the effects on growth and protein synthesis which are ordinarily seen in response to testosterone itself.

S. Cohen (Vanderbilt University) reported that the epithelial growth factor stimulates synthesis of ribosomal RNA, polysome formation, and protein synthesis, but not DNA synthesis in epidermis of chick embryos. Similar claims were reported for the nerve growth promoting factor (R. Levi-Montalcini and P. U. Angeletti, Washington University and Institute Superiore de Sanita, Rome, Italy, respectively).

Maureen Owen and Patricia Bingham (Churchill Hospital, England) extended this interpretation to parathyroid hormone, concluding that the initial effect of the hormone on both osteoblasts and osteoclasts is on nuclear RNA. Production of cytoplasmic RNA after stimulation of osteoclasts by parathyroid extracts (PTE) reaches a maximum of three times the control value by about 12 hours after PTE administration. Uptake of leucine and of glucosamine in the osteoclasts was also stimulated by PTE. This appeared to be correlated with the increased production of cytoplasmic RNA.

Thyroid-stimulating hormone (TSH) added to organ cultures of thyroid glands taken from 21-day-old fetal rats increased ^{32}P incorporation into total RNA (B. Nataf, University of Paris, France). Nataf suggested that one function of TSH might be to stimulate the synthesis of RNA that acts in the formation of thyroid proteins.

The idea implied in all these studies, that the primary action of hormones on developing target structures is the stimulation of some transcriptional event manifested as sudden changes in RNA synthesis, was also advocated by Tata (Medical Research Council, London). He cited evidence that thyroxine initiates metamorphosis through stimulation of RNA synthesis mainly of the ribosomal type. On the other hand, the possibility that thyroid hormone intervenes in protein synthesis on the level of translation rather than transcription was proposed by Geel and Timiras (University of California) and by Balazs and collaborators. Their hypothesis was based on measurements of RNA metabolism of cerebral cortex of hypothyroid rats. Conversion rate of (6-^{14}C)-

orotic acid into the RNA of the nuclear and other subcellular fractions, was unaffected by neonatal thyroidectomy, thus suggesting that the effect of thyroid deficiency on protein synthesis during early development may occur at the level of translation of the genetic message, rather than at the level of transcriptions.

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Fetal Growth and Development

A symposium on Fetal Growth and Development was held in San Diego, 16–20 November 1968, and sponsored by the Department of Pediatrics of the University of Wisconsin Medical Center with the cooperation of the Johnson and Johnson Pediatric Institute. It was planned by Harry A. Waisman and George R. Kerr of Wisconsin.

Benirschke discussed the relationship between the pathological development of the placenta and fetal malformations. He presented a number of examples, such as (i) the transfusion syndrome in monozygotic twins, where a shunt develops between the umbilical artery of one twin to the vein of the other; (ii) the frequency of harelip and cleft palate in human monozygotic vis-a-vis dizygotic twins. In the discussion it was pointed out that little is known about either the anatomy or the function of human placentas with chromosomal abnormalities. Villee summarized a variety of biochemical data pertaining to enzymatic differentiation in the fetus and the placenta. He spoke of "biochemical senescence" in which there is a decline of oxygen utilization, glycogen content, and a changing pattern of glucose metabolism. Winick discussed the cellular growth of various organs in the fetus and the placenta in terms of hyperplasia versus hypertrophy. The cellular growth of most organs, as measured by the total DNA content, ceases before birth is reached. Moog reviewed our knowledge of chemical embryology by discussing three enzymes. The first enzyme was tryptophan pyrrolase whose burst of activity is associated with a separation from the mother; the second, phosphopyruvate carboxylase, increases its activity remarkably soon after birth; a third example concerned the mysteries of the five LDH isomers in various species, each of which appears to have a different pattern. Hon focused attention upon the problem of continuous monitoring of the fetal heart by electrocardiography. Ramsey discussed the current techniques of placentography; the major part of her presentation consisted of a motion picture which was a beautiful demonstration of the maternal uteroplacental circulation during relaxation and contraction utilizing cineradioangiography. Diczfalusy of Sweden told of the fetal-placental endocrine unit, a concept which has been recognized within the past 5 years, and which helps explain why there is very little de novo synthesis of steroids from acetate; rather, the circulating sterols and steroids are utilized. Mèndez-Bauer of Uruguay summarized the effects of hypoxia on the fetus with particular reference to the Type II dips, or delayed deceleration, following uterine contractions. Nadler described how removal of amniotic fluid obtained in the middle trimester can be used to detect genetic malformations in the developing fetus. The fluid has also been used for isolation of viruses. The desquamated fetal cells may be used for cytological study, such as the sex chromatin which is of value in the prognosis of hemophilia carriers or they may be used as biochemical markers or for the cultivation of cells. Rudolph presented his methods of studying the fetal circulation in utero in the sheep. He developed a technique of injecting plastic microspheres with radioactive labels into varying portions of the fetal circulation to show that the umbilical flow, which constitutes 40 percent of the cardiac output, always falls when the fetuses are exteriorized. Lucey discussed the pigments of the amniotic fluid in relation to erythroblastosis, and pointed out the need for accurate diagnosis of hydropsfetalis in utero. Grumbach described the endocrine control of fetal growth and that there is an inherent capacity of an embryo or fetus to grow even though most or probably all of its endocrine organs are missing or quiescent. Dancis reviewed the processes of the placental transfer of sugars, amino acids, sodium and potassium, estrogens, and free fatty acids. He discussed the various methods of study which would be useful in further elucidation of membrane transport mechanisms in general. Silverstein presented the immunologic development of the lamb fetus, which is capable of responding to a selected antigen by the production of antibodies, and from that time on into the postnatal period a sequential achievement of competence occurs depending upon a hierarchal order of antigens. Sinclair told about the energy requirements for growth and differentiation, and what role O_2-utilization plays in the recovery from malnutrition. Sever described the significance of viral infections which are known to affect embryonic development of man, such as rubella, cytomegaloviruses, and toxoplasmosis. Intrauterine fetal malnutrition was discussed by Gruenwald, who told that interference with nutrition to the fetus is the result of deficiencies of the maternal uteroplacental circulation, rather than problems with placental transport. The subject of fetal malnutrition was continued by Naeye, who noted that cell size and cell numbers are actually more reliable indicators of malnutrition than organ weights or body weight. James reviewed the effects of acute asphyxia upon the brains of neonatal monkeys. Sontag described his early studies of recording types of fetal activity and fetal behavior in relation to subsequent personality and adult behavior. Waisman reported experiments on the effects of hyperaminoacidemia on fetuses of the rat and monkey. High phenylalanine dietary intakes by the mother led to marked increases of phenylalanine and tyrosine levels in the fetus resulting in mental retardation, even though the neonates received a normal diet. Hahn presented data on lipid metabolism by fetal rat tissues, both in vitro and in vivo, and noted that rats weaned prematurely were predisposed to hypercholesterolemia in later life. Drillien from Scotland noted that the brain may be permanently affected if nutritional restrictions are imposed at a time when it is undergoing a period of rapid myelination. Van den Berg closed the meeting by discussing the statistical approach to fetal and perinatal growth. Page summarized the conference by pointing out the gaps in our knowledge and the needs for some new areas of investigation in fetology from which future information could be applied to decrease fetal wastage and abnormal infants.

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