ON THE EXISTENCE OF A FOURTH HORMONE, THYREOTROPIC IN NATURE, OF THE ANTERIOR PITUITARY.

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RECENT investigation of the endocrine function of the anterior lobe of the pituitary has disclosed results which have converted earlier reasonable expectation into demonstrable fact. It is now established that extracts of anterior lobe, prepared in different ways, can produce the following diverse effects:

1. Excessive growth leading to gigantism.
2. Inhibition of oestrus.
3. Induction of oestrus.
4. Increase in the basal metabolism rate.
5. Metamorphosis in the neotonous amphibia.

The multiplicity and diversity of these effects naturally led to the assumption than more than one anterior pituitary hormone must be responsible for them, and so it is that some have attempted to distinguish a growth hormone and a sex or gonadotrope hormone (Evans and Simpson, 1928), whilst others have sought to analyse the latter into a hormone that promotes follicular development in the ovary and another which induces luteinization. By some it has been assumed that the growth hormone is antagonistic to the sex hormone—the oestrus-producing agent (Evans and Simpson); and that this antagonism explains away the fact that certain extracts containing the growth hormone actually inhibit the oestrus. Others have postulated that there are two oestrus-influencing factors involved, one inciting it, the other inhibiting it. The present writers, on the basis of their own experiments, have elsewhere succeeded in reconciling certain of these apparently conflicting opinions. They postulate the existence of two gonadotropic hormones of the anterior pituitary, Rho 1 and Rho 2 respectively, and refer the difference in gonadotropic effect to quantitative and qualitative differences in the preparation of these factors in any given anterior pituitary extract. Rho 1 is the oestrogenic factor, Rho 2 the kyogenic. The first incites in the ovary the first phase of activity, characterized by the elaboration of oestrin which leads to the phenomenon of cornification of the vaginal epithelium. Rho 2 evokes the second phase of ovarian activity, characterized by the activation in the ovary of the lutein tissue which elaborates substances that evoke the nidational potencies in the uterus, and mucification, the development of high mucous epithelium, in the vagina.

Evans and Simpson have shown that the growth hormone is not contained in human placenta, whilst Wiesner and Crew have demonstrated the presence of Rho factors therein. Since it has been found possible to obtain extracts which contain both Rho factors, but which do not contain the growth hormone, and since it is possible to extract Rho factors with acid as well as with alkaline media, whereas the growth hormone is not present in acid extracts (Hewitt), it follows that the Rho factors and the growth hormone are distinct and separable.

It has been shown by Adler, Allen, Spaul, and others, that certain anterior lobe extracts invoke metamorphosis in the neotonous amphibia. The question thus arises as to which of the known hormones of the anterior pituitary is responsible for this effect, for if none of these produces metamorphosis, then it becomes necessary to postulate the existence of yet another anterior lobe hormone responsible for this phenomenon. It is established that thyroxine can induce metamorphosis, but it is most unlikely that the hormone of the anterior pituitary which produces metamorphosis is identical with this substance (Spaul).

Since the feeding of large amounts of anterior lobe can promote growth in the axolotl in the absence of metamorphosis, it would seem that the metamorphic factor is not the same as the growth factor. It is of interest to note that Spaul has produced evidence which shows that there exists a different regional distribution in the pituitary of this growth factor and of the metamorphic factor.

In order to examine the question as to whether or not Rho 1, Rho 2, or the growth hormone was identical with the metamorphic factor, the following experiments were planned. To different sets of young axolotl, three in each, the following substances were administered, 0.5 c.cm. being given daily over a period of a month: (1) thyroxine; (2) extracts of human placenta prepared by the method of Wiesner (1930) containing Rho 1 and Rho 2, and known to be highly oestrogenic; (3) extracts of anterior lobe containing the growth factor prepared by the method of Evans and Simpson. Only those animals which received thyroxine metamorphosed. Since it has been shown that the presence of posterior lobe derivatives in an anterior lobe preparation leads to a retardation of the metamorphic effect, it might well be that in these extracts posterior derivatives were present, and the failure of Rho factors to induce metamorphosis might be due to a secondary cause.

But a further series of experiments permitted us to arrive at certain definite conclusions. It is to be noted that the active extracts of Spaul and others—for example, of Uhlenhuth—are prepared by boiling anterior lobe in diluted acetic acid for ten minutes. Now it had been observed that the Rho factors are completely destroyed if boiled for more than three to five minutes. In accordance with these observations we found that extracts prepared by Spaul's method and injected in various doses into immature mice proved to be inactive. In no case did cornification or mucification of the vaginal epithelium follow; though extracts prepared with acetic acid without boiling definitely activate the ovary and thus lead to significant changes in the vaginal epithelium. It follows, then, that the extracts prepared by Spaul's method do not contain the Rho factors. Boiling also destroys the growth factor, as was found by Evans; and it is of interest to note that Spaul states that the use of alkaline media is detrimental to the preparation of the metamorphic factors, though this has been shown to be the best method for the extraction of the growth factor (Evans, Hewitt, Putnam, Teel).

There would seem, then, to be no escape from the conclusion that there must be at least four hormones elaborated by the anterior pituitary—the growth factor, two gonadotropic factors (presently called Rho 1 and Rho 2), and a metamorphic factor, which we think provisionally should be given the name of Theta.

Since metamorphosis is produced in the neotonous amphibia by the mammalian pituitary, it follows that the theta factor is present, even in those forms in which metamorphosis does not occur. What, then, is its role?

Certain observations suggest that this theta factor is thyreotropic in its action, being the activator for the release of thyroid development and activity. It has been shown by Uhlenhuth and Schwarzbach that the thyroid of an animal metamorphosed by anterior pituitary undergoes that series of developmental changes which are characteristic of normal metamorphosis. Moreover, it has been noted by Allen and others that the thyroid of hypophysectomized animals becomes atrophied. It is true that Hogben and Spaul have both recorded instances of metamorphosis induced by anterior pituitary extract in the thyroidless individual, but it would appear that this metamorphosis was not complete in the first author's cases, and, as Uhlenhuth has pointed out, it is not yet decided that in these experiments the thyroid was completely removed. This view is supported by certain other facts. Extracts of anterior lobe which induce metamorphosis also provoke a rise in the metabolism of the frog (Uhlenhuth). This is as would be expected if the extracts contained substances which stimulated the thyroid, or if the metamorphic factor in the anterior pituitary were thyreotropic in action. Moreover, it has been reported by Smith

that the degenerative changes in the thyroid following hypophysectomy are repaired following implantation of anterior lobe substance.

Uhlenhuth would seem to be justified, therefore, in ascribing the metamorphic effect of anterior lobe extracts to the presence of an activator of the thyroid therein. Be that as it may, our own experiments show that not only is the metamorphic factor not identical with the growth hormone—as has been concluded by Spaul—but that it also differs from the gonadotropic hormones. It may be assumed, therefore, that a fourth anterior pituitary hormone (theta) exists. Further experiments will have to show whether it is thyrotropic, as it seems to be.

INTRAVENOUS PYELOGRAPHY.

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EVER since Ehrlich in 1906 produced salvarsan, scientific workers all over the world have been looking for an anti-septic which, when injected intravenously, shall be harmless to the body tissues and yet be able to kill micro-organisms. Their ideal has never yet been realized, but many useful synthetic products have resulted from their search. Uroselectan (sodium 2 oxy-5-iod pyridine-N-acetic acid) is one of these.

For some years prior to 1928 Professor Binz and Dr. Raeth had been experimenting with an organic compound of iodine selectan-neutral, which, it was hoped, would be of use in coccal injections of the biliary and urinary tracts. Erbach, moreover, had shown that this compound, with a high molecular weight, was excreted in part by the kidneys, and naturally these facts suggested that it might throw a shadow of the urinary tract on radiographic examination.

Experiments to this end were carried out by Swick¹ in Lichtwitz's clinic, with only more or less satisfactory results. The clarity of the shadows was variable; they were not sufficiently dense for reproduction, and it was found that, whether the compound was administered by the mouth or intravenously, there was a good deal of individual variation in tolerance. Furthermore, a large number of reactions, such as headache, sickness, and vomiting, were recorded. For these reasons it was later on suggested by Swick and von Lichtenberg that sodium glycin should be substituted for a methyl group, and the solubility of the compound increased by a slight reduction of the iodine content. This work was carried out by Binz and Raeth,² and the compound called uroselectan was the result. The formula of the preparation is shown below.

Chemical and Physical Properties.

It is neutral, soluble in water to more than 50 per cent., and contains 42 per cent. of iodine. Its opacity to x rays is shown in Figure 1. It is an extremely stable chemical compound, which can be introduced into the blood stream in relatively large amounts. If deductions from animal experiments³ are reliable, it is possible to give an intravenous dose of 180 grams to a man weighing 50 kilos, but for clinical purposes 40 grams is all that is necessary to

produce perfectly clear-cut shadows. This amount is dissolved in 80 c.cm. of redistilled water, filtered twice, and autoclaved for twenty minutes. The result is a heavy brown solution, which, however, contains no free iodine. Experiments have been made with a view to sterilizing it by boiling, and a solution boiled for three hours has been used with no ill effects. For children the dose is proportionately smaller. Up to the age of 6 we inject 10 to 12 c.cm. of the 40 per cent. solution; from 6 to 10 years, 20 to 40 c.cm.; and from 10 to 15 years, 50 c.cm.

Technique.

The patient is prepared for the x rays in the usual way, and a preliminary radiogram is taken. The solution at body heat is then injected slowly into a vein, preferably in two equal doses, at an interval of from two to three minutes. For routine purposes radiograms are then taken, a quarter of an hour, three-quarters, and one and a quarter hours after injection.

Generally speaking, the rate of excretion bears a direct relationship to the renal function, as determined by the ordinary tests, and after a quarter of an hour in cases with a good renal function, not only is the whole urinary tract clearly visible, but there is a definite increase in the normal renal shadow. This latter is naturally more easily appreciated in the film than in a reproduction. The standard of radiographic technique demanded is high. The

FIG. 1.—1. Uroselectan. 2. Half uroselectan and half 2 per cent. urea. 3. Half uroselectan and half 4 per cent. urea. 4. 2 per cent. urea. 5. Water. 6. 20 per cent. sodium iodide.

shadow thrown by the drug as it is excreted by the kidney is not quite as dense as that ordinarily obtained by the introduction of media through ureteric catheters, and it is therefore of paramount importance that the maximum contrast should be obtained upon the radiogram. For this reason we use a low penetrating ray such as is emitted from a tube excited by a maximum potential of 65 kilovolts peak.

A Potter-Bucky diaphragm fitted with a pressure band in conjunction with double intensifying screens is used, the focal plate distance being thirty inches. With a milli-ampere of 60, the exposure will vary with the bulk and build of the patient, and will ordinarily be from one to five seconds. The radiograms are developed for five minutes in a standard developer heated to 65° F. precisely. The radiograms when completed are examined in lanterns of equal illuminant power. No variation from the normal procedure is permissible.

It must be pointed out that important information is obtained by comparing radiograms of a series, and any variation in the relative density of the drug-laden urine must be independent of variations in radiological technique. Several of our patients were radiographed at three-minute intervals, and in one patient radiograms were obtained at intervals of five seconds. Should such procedures become general, and the results of more than academic interest, it may be necessary to devise serial changing cassette holders working in conjunction with a Potter-Bucky diaphragm. The cinematograph may in the future give the perfect serial visualization of kidney function.