BRITISH MEDICAL JOURNAL

LONDON SATURDAY NOVEMBER 17 1951

THE EXPERIMENTAL APPROACH TO THE PROBLEM OF DIABETES MELLITUS*

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It was said of Sydney Ringer that "clinical medicine was his profession and scientific research his recreation." As one who is a biochemist with an interest in the relationship of the basic sciences to medicine I am particularly honoured to deliver a lecture in memory of a distinguished clinician whose laboratory researches exerted such a fundamental and fruitful influence upon biological science.

The accidental discovery by Mering and Minkowski in 1889, that experimental removal of the pancreas from a dog induces a condition somewhat resembling human diabetes mellitus, supported the simplest view of the aetiology of the human condition—namely, that it was the result of pancreatic deficiency. The pathological observations of Opie and others, based as they were on the earlier histological findings of Langerhans, provided some evidence to this end, and the discovery of insulin by Banting and Best in 1921 still further strengthened this view. Indeed, the unparalleled importance and efficiency of insulin as a therapeutic agent throws into strange contrast our long-continued ignorance of the aetiology of diabetes and of the detailed mechanism of action of insulin.

During the years since the discovery of insulin the idea that human diabetes is always the result of a primary deficiency in the capacity of the pancreas to secrete sufficient insulin for normal needs has become increasingly untenable. For instance, in a small proportion of cases the insulin requirements of the human diabetic may rise to extraordinary high levels, sometimes up to 4,000 units a day for a short time. Such a degree of resistance to the action of insulin can exist without obvious infectious complications, but is happily seldom long maintained.

Arguments based on the insulin requirement of the depancreatized dog and applied to human diabetes are quantitatively dangerous, but they suggest quite a different order of requirement for insulin of the depancreatized human, and in recent years direct observations on human patients depancreatized for therapeutic reasons suggest that their insulin requirement is unlikely to exceed 60 units a day (cf. Waugh *et al.*, 1946). These observations indicate that, in some

instances at least, the existence of human diabetes must be associated with factors exerting an action which is antagonistic to that of insulin. That the amount of insulin needed to control human diabetes may vary significantly with the nature of the diet and with other conditions has been stressed, particularly in this country by Himsworth (1949). These observations further emphasize that insulin deficiency alone may not determine the severity of the condition in human diabetes.

The Diabetogenic Influence of the Pituitary Gland

The path leading to a partial answer to the question of the nature of some factors antagonistic to insulin was indicated by the fundamentally important observations of the Argentinian investigator B. A. Houssay, who, with his colleagues, found that removal of the pituitary gland, or of its anterior lobe only, substantially alleviated the severity of pancreatic diabetes in a variety of species. A corollary of these findings was the induction of a diabetic condition in normal animals by the administration of anterior pituitary extract, and the first striking demonstration of the diabetogenic action of anterior pituitary extract came from the laboratory of H. M. Evans under conditions which we now see to be highly significant.

Evans and his colleagues (1932), with the aim of producing giant animals, treated puppies daily for many months with a growth-promoting extract of anterior pituitary extract. After six to eight months of treatment some of the animals had become ill and were found to be diabetic. This diabetes was associated with an infectious condition of the skin, and the condition persisted for some time after the cessation of pituitary treatment. About this time (1932) Houssay and his pupils succeeded in inducing a diabetic condition in normal and in partially depancreatized animals by a few days of daily treatment with crude anterior pituitary extract (see Houssay and Biasotti, 1932).

We were able to confirm these findings, so far as the diabetogenic activity of crude pituitary extracts was concerned, in intact dogs and cats treated daily for a few days (Young, 1936, 1937). Subsequently we have used intact animals, first the dog and more recently the cat, as test animals for diabetogenic activity, with satisfactorily consistent results, but Houssay and his

*The 19th Sydney Ringer Memorial Lecture, delivered at University College Hospital Medical School, London, on March 16, 1950.

colleagues, and also many workers in the United States, have apparently found inconsistent results with intact animals, and have consequently employed dogs or cats from which up to 87% of the pancreas has been surgically removed at a preliminary operation. This operation induces a condition in which the animal very readily exhibits glycosuria after the administration of anterior pituitary extract, but it is one in which the animal's response may be less specific for what we define as the diabetogenic factor of the anterior pituitary gland—that is, a substance whose administration leads to diabetes in an intact animal. Moreover, some partially depancreatized animals may exhibit glycosuria when given control treatment such as the administration of inert protein material. We have had no difficulty in obtaining consistent results with the intact adult dog or cat as test animal and cannot readily account for the difference between our results and those of our colleagues abroad.

While, in our hands, the dog and the cat easily and quickly become diabetic when given daily injections of ox anterior pituitary extract for a few days, many other species of animal do not respond in this way. In fact, the only species which we find to respond consistently to such pituitary treatment by exhibiting diabetes are the dog, the cat, and the ferret. The strains of rabbit we have used do respond, but not consistently, while those convenient laboratory species the rat, the mouse, and the guinea-pig do not become diabetic when given large doses of an extract which is highly diabetogenic in the adult dog and cat. The intact rat, even in an elderly senile condition, always responds to such pituitary treatment, not with diabetes, but with an increased rate of growth. In the laboratory of Professor C. N. H. Long at Yale University the strain of rats is such that partially depancreatized diabetic animals show an increase in the severity of the diabetes when treated with appropriate pituitary extracts, but the strains of rat available to us differ from those in Yale, since we find that treatment of diabetic animals with diabetogenic pituitary extract induces no consistent increase in the severity of the diabetes.

With the intact cat as test animal we found (Cotes, Reid, and Young, 1949) that purified growth hormone is highly diabetogenic, although we could not rule out the possibility that even the most highly purified preparations of growth hormone available to us were contaminated with traces of a substance or substances essential for or contributing to the diabetogenic activity of the growth hormone. Recently Houssay and Anderson (1949), with the partially depancreatized cat or dog as test animal, have found that hyperglycaemia follows the administration of growth hormone, but in cats from which 75% of the pancreas had been surgically removed they administered as much as 25 mg. of purified growth hormone per kilogram of body weight per day in demonstrating this effect. On the other hand, we found (Cotes *et al.*, 1949) that the intact cat would respond with substantial glycosuria to a dose of growth hormone less than 1 mg./kg./day.

Although the diabetogenic activity of pituitary adrenocorticotropin has been demonstrated in the intact rat (Li, Ingle, and Evans) and in the normal human (Conn) we have so far failed to demonstrate a diabetogenic action of adrenocorticotropin in the intact cat in our laboratory, and Dr. E. Reid has administered up to 50 mg./kg./day to the cat without inducing glycosuria.

In the dog from which 83–87% of the pancreas had been surgically removed Houssay and Anderson found that 50 mg./kg./day of purified adrenocorticotropin induced a moderate rise of blood sugar in only one dog out of four treated ones, in an experiment lasting four days.

Although adrenocorticotropin must be regarded as a diabetogenic agent under some conditions we believe that the diabetogenic activity of the ox pituitary extracts we have used to be largely attributable to their growth hormone content, although the possibility that a small dose of adrenocorticotropin is present and exerting a co-operative action cannot be ruled out at this stage.

Growth and Pituitary Diabetogenesis

The observed diabetogenic action of growth hormone in the intact adult dog and cat made particularly interesting the results of long-term treatment of the kitten and puppy with diabetogenic-growth-promoting pituitary extracts. As is shown in Table I, large doses of diabeto-

TABLE I.—*Diabetogenic Action of Anterior Pituitary Extract (The Values are those Per Kg. of Initial Body Weight)*

	Adult Dog	Adult Cat	Puppy	Kitten
Extract injected daily (mg. equivalent of growth hormone)	1.5	0.6	6.0	3.75
Days before onset of glycosuria	4.7	5.6	—	a
First day:				
Glycosuria (g.)	1.97	1.93	—	—
D/N	1.37	1.23	—	—
Second day:				
Glycosuria (g.)	4.63	2.17	—	—
D/N	2.91	1.74	—	—
Net increase in growth rate (g./day)	10.0 (5 days)	8.9 (6 days)	19.1 (21 days)	16.3 (21 days)

genic anterior pituitary extracts induced not diabetes but extra growth in puppies and kittens over a period of three weeks, and even in experiments lasting many months the treated kittens and puppies did not become diabetic. Thus it appeared that if the test animal was able to grow, then growth rather than diabetes would occur. Such a view would account for the fact that the intact rat, whose epiphyses never close and which is able always to respond to the administration of growth hormone by growing almost indefinitely, never, in our experience, exhibits diabetes as the result of treatment with growth-promoting anterior pituitary extract. It was therefore of further interest to know whether diabetes would subsequently develop in the long-treated kittens and puppies at a time when they might be regarded as having reached adulthood. So far as the puppy was concerned we did find that a proportion of the long-treated animals developed diabetes (Young, 1941, 1944; cf. Evans *et al.*, 1932; Evans, 1933). Nevertheless in experiments with over 50 kittens not one became diabetic even when the treatment was continued until after the animal had reached adulthood. In this respect the cat which has grown naturally to adulthood differs from the animal whose growth has been hastened by treatment with growth hormone.

We have studied five of the long-treated puppies which subsequently became diabetic. In some of them striking joint changes developed during the early weeks of the experiments, but these changes exerted no obvious course on the growth of the animals, and no differences were observed between the metabolic response of those animals which showed such changes and those which did not. In the long-treated puppies which became

FIG. 1.—Data for dog 124 during the period when protamine-zinc insulin and diabetogenic anterior pituitary extract were given daily simultaneously. The injections were begun on day 223, which is indicated by the vertical dotted line. The dose of extract (5 ml. per day—approximately equivalent to 30 mg. growth hormone per day) was that which induced growth without diabetes during the first 19 weeks of continuous daily treatment. The food intake was unlimited through the experiment.

diabetic the growth response to the pituitary treatment diminished *pari passu* with the development of the diabetic condition, and ultimately the animals stopped growing or even began to lose weight. In these animals further growth could then be induced by the administration of insulin simultaneously with the pituitary extracts (Figs. 1 and 2), although the amount of insulin needed was very large indeed (Fig. 1). We may have here a counterpart to the cases of insulin-resistant diabetes occasionally met with in human beings.

It was clear from these experiments that the diabetogenic action of growth hormone could be converted to a growth-promoting one in the dog if enough insulin were administered with the pituitary extract. This raises two important questions: (1) Does treatment with growth hormone normally induce an enhanced rate of

secretion of insulin by the pancreas? (2) Were the pancreases of the treated puppies, during the period before the appearance of the diabetes, secreting an excessive amount of insulin as a requirement for the exhibition of growth at this stage of the experiment?

There is much evidence in support of an affirmative answer to the first question (e.g., Milman and Russell, 1949). It may be that under the influence of anterior pituitary growth hormone a fall in the concentration of insulin in the blood occurs, without a corresponding rise of blood-sugar level, and that this fall provides a stimulus for further secretion of insulin by the pancreas. The answer to the second question is probably also positive, although the alterations in carbohydrate metabolism which occur as the young animal grows are still little known (cf. Langfeldt, 1920), and changes in the activity or reactivity of enzyme systems as age advances may be of greater significance than alterations in the rate at which a hormone such as insulin is secreted.

The general conclusion to be drawn from these observations is that the diabetogenic action of growth hormone is regularly demonstrable only in certain adult carnivorous animals, and that in the young of even these species growth rather than diabetogenesis occurs in response to treatment with growth hormone. The association of acromegaly with diabetes, and the finding of Priscilla White that diabetic children are often over-height, are observations in good agreement with this general view.

If, then, growth and growth-hormone-induced diabetes are alternative responses to the same general type of metabolic stimulus, it was of interest to know if the diabetogenic action of growth hormone in intact adult animals could be regarded as the result of a growth stimulus which had become abortive in the adult animal because the adult could not grow indefinitely under the influence of the stimulus. For the elucidation of this question the metabolic balance of dogs and cats treated with anterior pituitary growth-promoting extracts was investigated.

Energy Balance in Idiohypophyseal Diabetes

It is convenient to define idiohypophyseal diabetes as the condition which exists during a period of anterior pituitary treatment (or of naturally occurring pituitary over-activity) in order to distinguish it from metahypophyseal diabetes, the condition which persists under suitable conditions after the cessation of excess pituitary activity.

Now, when intact adult cats and dogs are given subcutaneous administrations of growth-promoting anterior pituitary extract daily for a week or more, the initial response, lasting three to five days, is a rise in body weight associated with nitrogen retention; the fasting blood-sugar level usually does not rise during this period, but may show a tendency to fall slightly. After three to five days the blood-sugar level rises and glycosuria, sometimes accompanied by ketonuria, appears. At this time the upward trend of the weight curve diminishes or subsides, though the retention of nitrogen continues. One effect of growth-hormone administration is an increase in the appetite, and the rise in food intake contributes to the elevation of the body weight. Nevertheless, the rises in body weight and nitrogen retention

FIG. 2.—The influence of protamine-zinc insulin plus diabetogenic pituitary extract (5 ml. per day—approximately equivalent to 30 mg. per day of growth hormone) on dog 125. The daily injections were begun on day 280, which is indicated by the vertical dotted line. The food intake was controlled from day 270 onwards.

are observed even if the animals receive daily throughout the experiment a constant amount of food sufficient just to maintain a steady body weight before pituitary treatment begins (Young, 1945). Similar observations with respect to nitrogen retention were made with dogs receiving insulin as well as anterior pituitary extract when the food intake was unlimited (Fig. 1), or when the food intake was constant throughout the experiment (Fig. 2).

In all these instances analysis of the tissues of the liver and muscle showed that these were essentially normal in composition, and it appeared that the rise in body weight was associated with a deposition in the body of protein-containing tissue of the composition of muscle, although in some instances the amount of nitrogen retained was more than could be accounted for by the rise in body weight observed (Young, 1945). In intact rats treatment with the same diabetogenic extract did not, of course, induce diabetes but growth only, this growth being associated with a retention of nitrogen even though the food intake was kept constant (Young, 1945; see also Lee and Schaffer, 1934). In the rat the rise in body weight was associated with an increase in the weight of many but by no means all of the skeletal muscles (Greenbaum and Young, 1950).

Direct analysis of the carcasses of the treated rats showed that the deposition of protein-containing tissue was accompanied by a loss of fat, and similar conclusions were drawn from the results of the experiments with dogs and cats (Young, 1945). The energy balance of dogs and rats, treated with anterior pituitary extract alone during an experiment involving a constant daily food intake throughout, is indicated in Fig. 3. Similar data for dog 124, which received insulin simultaneously with the pituitary extract and whose food intake was unlimited, are illustrated in Fig. 4, together with results for dog 125, which was given insulin together with the pituitary extract but whose food intake was limited to that just sufficient to maintain constant body weight, with appropriate insulin treatment before the daily administration of anterior pituitary extract was begun.

It will be seen that the combustion of extra fat contributed additional energy at a time when energy was being lost by the deposition of new protein-containing tissue, and, in the experiments with the dogs, by the excretion of sugar in the urine. The rise of body weight in the experiments with a constant food intake throughout was to be attributed to the fact that approximately 9 kg. cal. of energy is provided by the oxidation of

FIG. 3.—The data are mean values—for one week in the case of the dog and for nine weeks in the case of the rat. The food intake was constant throughout the experiment and was just sufficient to maintain a constant body weight before the experiment began. The dogs were normal intact animals.

FIG. 4.—The data are mean values—for five weeks in the case of dog 124 and for nearly three weeks in the case of dog 125. The food intake of dog 124 was unlimited (see Fig. 1), while that for dog 125 was controlled (see Fig. 2).

1 g. of depot fat, while little more than 1 kg. cal. of energy is lost when 1 g. of muscle tissue, containing 70% of water, is oxidized. Thus under isocaloric conditions the body weight could rise by nearly 8 g. for each 1 g. of depot fat oxidized. A comparison of the balance sheet for dog 124, whose food intake was unlimited, with that for dog 125, whose food intake was kept constant throughout the experiment, is given in Fig. 4.

Treatment with the diabetogenic pituitary extract, together with enough insulin to suppress ketonuria, induced an increase in appetite of such magnitude that the calories from the extra food consumed were more than sufficient to cover the loss of energy resulting from the excretion of glucose and deposition of new tissue induced by treatment with the extract. In some instances it appears that the rise of appetite induced by the administration of growth hormone may be sufficient to induce a deposition of fat quantitatively greater than the extra loss due to the enhanced rate of fat oxidation, with the result that the animal may deposit protein and fat simultaneously.

The overweightness of the majority of human beings who developed diabetes is well recognized. The possibility that this excessive weight might result from a long-standing oversecretion of pituitary growth hormone, too small overtly to induce the skeletal changes and other stigmata of acromegaly, is an attractive one. In the human being the rise in appetite which would be expected to follow hypersecretion of growth hormone might well compensate, or more than compensate, for the increased rate of oxidation of depot fat. It follows, therefore, that if the overweightness of human pre-diabetics was the result of pituitary hypersecretion the excess weight would not be ascribable to adiposity alone, but would depend in part upon the deposition of protein-containing tissues. Now the deposition of extra protein-containing tissues tends to raise the specific gravity of the body as a whole (Behnke *et al.*, 1942; Young, 1945), and the possibility that the determination of the specific gravity of patients suffering from overweightness might lead to findings of significance, particularly with respect to the aetiology of diabetes, is one for serious consideration.

Antagonism between Insulin and Growth Hormone

The possibility that human diabetes may result from an antagonism between insulin and growth hormone emphasizes the importance of the views of C. F. and G. T. Cori concerning the opposing influence of these two hormones with respect to the activity of the enzyme hexokinase. This enzyme catalyses the formation of glucose-6-phosphoric acid from glucose and adenosine triphosphoric acid (A.T.P.), and it may well be that the formation of glucose-6-phosphate under the influence of hexokinase constitutes an obligatory initial step in the utilization of glucose for metabolic purposes in general in the animal body.

As was first demonstrated by Gemmill (1940), the isolated diaphragm of the rat will take up glucose from the surrounding medium, and the rate of uptake is enhanced by the addition of insulin to the system *in vitro*. It may be that the limiting factor with respect to the uptake of glucose by the muscle of the diaphragm under these conditions is the activity of its hexokinase system, and that insulin is indeed capable of increasing the activity of the hexokinase *in vitro*, although such is not the only possible interpretation of the observed results. C. F. Cori, however, does believe that under suitable conditions the rate of glucose uptake by rat diaphragm *in vitro* is a safe index of the hexokinase activity of the muscle; and if this assumption is correct the findings of the Coris and others support the view that insulin enhances hexokinase activity while growth hormone depresses it.

I should like to mention the possibility that growth hormone is not acting directly on the hexokinase but influencing the activity of this enzyme by indirect means. This could be so if two enzymes were concerned, these two competing for a limited amount of A.T.P. at the cell surface. The activity of one of these enzymes, hexokinase, would be enhanced by the presence of insulin, while that activity of the others concerned with the transfer of amino-acids across the cell membrane might be enhanced in the presence of growth hormone. The possibility that certain adrenal steroids catalyse the utilization of A.T.P. for other translocatory processes—for example, those involving cations—might also be considered.

Metahypophysial Diabetes

Metahypophysial diabetes persists, in the intact dog and cat, after the cessation of a short period of pituitary treatment (Young, 1937, 1948), and its persistence is almost certainly to be ascribed to the lesions which are found in the islets of Langerhans in such instances. The existence of metahypophysial diabetes in animals indicates the possibility that in the human being a short period of pituitary hyperactivity with respect to growth-hormone secretion could result in permanent damage to the islets of Langerhans of the pancreas and thus induce a persistently diabetic condition. Such a metahypophysial diabetes would not be of an insulin-insensitive type if the hyperactivity of the pituitary gland subsided completely, and indeed would find its direct though not primary origin in the pancreatic lesions alone.

Influence of Growth Hormone on Milk Production

Although it may seem at first strange that milk production is to be considered in a discussion of diabetes it is hoped that the reason for this will quickly become apparent. In 1938 Dr. S. J. Folley and I found that the galactopoietic activity (that is, the ability to enhance the milk yield of animals already secreting milk) of anterior pituitary extracts in the cow paralleled their diabetogenic activity in the dog (Folley and Young, 1938), and more recently we have shown that purified growth hormone is highly galactopoietic in cows in declining lactation (Cotes, Crichton, Folley, and Young, 1949). Neither prolactin nor adrenocorticotropin is galactopoietic under the conditions of our experiments, though these hormones may be of importance in the *initiation* of lactation. We believe that it is necessary, in this connexion, to distinguish clearly between the initiation of lactation and the stimulation of an existing milk flow.

Since growth hormone was found to be galactopoietic it was of interest to know how far the treatment of lactating rats with growth-promoting anterior pituitary extract would stimulate the growth rate of the mothers, and how far it would enhance the flow of milk and thus indirectly stimulate the growth of the offspring. The results in Table II show that the *gross* increase

TABLE II.—*Influence of Growth-promoting Anterior Pituitary Extract on the Growth of Nursing Rats and Their Litters (The Figures are Mean Results for Groups of 10 Rats Treated Daily for 14 Days)*

	Nursing Rats		Normal Female Rats	
	Saline-treated	Pituitary-treated	Saline-treated	Pituitary-treated
(a) Mothers:				
Initial weight (g.)	222	230	221	220
Final weight (g.)	235	271	222	263
Increase (g.)	13	41	1	43
Net increase (g.)	—	28	—	42
(b) Young:				
No. in litter	6.3	6.9		
Final weight of young (g.)	27.9	28.4		

in the weight of pituitary-treated mothers is about the same as that of pituitary-treated non-lactating female rats; but, since the control lactating rats rose significantly in weight while the control non-lactating rats did not, the *net* increase in weight of the pituitary-treated lactating rats was less than that of the treated non-lactating animals. The sucklings of the pituitary-treated

mothers were not significantly heavier than those of the control mothers at the end of this experiment, and thus there was no evidence of a galactopoietic effect of the extract under these conditions. One might say that when exogenous growth hormone was administered the growth of the mother took precedence over the secretion of extra milk under these conditions.

We have known for many years that pregnant or lactating dogs or cats do not become diabetic when given doses of diabetogenic anterior pituitary extract which are highly effective in inducing diabetes in normal cats or dogs; in fact, one of my earliest failures to produce diabetes with a potent anterior pituitary extract was that of a pregnant bitch (Young, 1938). It would be of great interest to know whether, in such animals, the growth stimulus is confined to the mothers or shared with the offspring, but our present data are insufficient to allow us to draw a definite conclusion on this point so far as the dog and the cat are concerned. For the rat, on the other hand, relevant observations have long been present in the literature. Thus Watts (1935) observed that the treatment of pregnant rats with certain partially purified preparations of the anterior pituitary growth hormone could induce not only a rise in the body weight of the mothers over that of control animals but also a statistically significant increase in the weight of the offspring. This excessive weight of the young from treated mothers was observed in animals for which the gestation time had not been prolonged by the treatment, although in some of Watts's experiments, and also in many of those of others, treatment with some preparations of growth hormone did induce a prolongation of pregnancy in the rat. Such a prolongation can be legitimately ascribed to contamination of the admittedly impure growth hormone with gonadotropins, and was not seen with more purified preparations of the growth hormone.

Cole and Hart (1938) observed that in the rat normal pregnancy stimulates the growth of the body of the mother, and that this extra weight was not completely lost either during lactation or subsequently. In our own experiments untreated lactating rats appeared to show, during the period of lactation, a significantly greater growth rate than did control non-lactating female rats (Table II), and this observation may be indicative of an enhanced secretion of growth hormone during the period of lactation. Our evidence, together with that of Watts (1935) and of Cole and Hart (1938), is in agreement with the view that during both pregnancy and lactation there is an enhancement of the rate of secretion of growth hormone by the anterior pituitary gland, at least in the rat.

With the identification of the growth hormone as an important diabetogenic agent the above considerations are possibly relevant to the observation of Miller, Hurwitz, and Kuder (1944), which has been adequately confirmed, that the tendency to produce large babies, and the high incidence of foetal mortality, found in diabetic women may often be present many years before diagnosable diabetes develops. If an enhanced secretion of growth hormone by the anterior pituitary gland is a factor of importance in the stimulation of foetal growth, and also, after parturition, in the stimulation of milk production, then excessive secretion of growth hormone, beyond the capacity of the tissues to respond adequately, might lead to diabetes as the pathological result of an overstimulated physiological process. It is of interest

to recall the classical experiments of Paul Bert in 1884 in which mastectomy of a pregnant goat led, at parturition, to the transitory appearance of a diabetic condition. Here the removal of the means whereby the physiological process could realize itself led to the pathological condition of a temporary diabetes.

If, in diabetic women, the large babies are to be ascribed to the action of an excessive secretion of growth hormone, I would suggest that the accidents of pregnancy in such women are more likely to be the result of other factors—perhaps an abnormality of secretion of pituitary gonadotropins

Conclusion

In a paper published some years ago (Young, 1947) I wrote: "Growth, including foetal growth, and milk production are processes requiring a special type of metabolic control in that they both necessitate the preservation from oxidization of foodstuff that would otherwise, in an animal in equilibrium, be oxidized. . . . Since growth, galactopoiesis, and diabetogenesis all involve a restraint on oxidative processes, it would not be surprising if there were some relationship between the hormonal mechanisms concerned with the control of these phenomena."

The evidence now at our disposal allows us to associate growth, including foetal growth, and milk production as phenomena whose development is stimulated by growth hormone. Excessive pressure on these processes in circumstances where the physiological stimulus cannot fully or properly realize itself can lead to the pathological outcome of diabetes, a condition in which the material preserved from oxidation but not utilized may be excreted in the form of sugar. In the human being a slightly excessive rate of secretion of growth hormone, insufficient to cause overt signs of acromegaly but lasting a long time, might lead to a prolonged but not excessive restraint upon the processes of carbohydrate and protein catabolism and oxidation, combined with the increase in appetite characteristic of growth-hormone activity, so that diabetes ultimately develops. In growth hormone we have a substance that may well be of importance in the aetiology of a serious proportion of cases of human diabetes mellitus.

I wish to express my thanks to the Medical Research Council for their continued support of my researches upon experimental diabetes.

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HYALURONIDASE SKIN SPREADING EFFECT*

AN ANALYSIS OF REPEATED MEASUREMENTS

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Hyaluronic acid has been demonstrated in man in the skin, umbilical cord, synovial fluid, vitreous humour, and some mesenchymal tumours. Its presence is inferred in connective tissue (Wislocki, Bunting, and Dempsey, 1947) on histological evidence and by observation of the effect of hyaluronidase. This enzyme system, found in some bacteria and in testicular extract, reduces the viscosity of, and hydrolyses, hyaluronic acid (Meyer *et al.*, 1937, 1940). The possible role of hyaluronic acid and hyaluronidase in connective-tissue diseases has been the subject of wide interest. Guerra (1946a, 1946b) claimed that the skin of patients with acute rheumatism gave enhanced spreading with the enzyme, and, further, that administration of salicylates inhibited this spread. Subsequently reported work (Harris and Friedman, 1949; Jaworski *et al.*, 1950) has not confirmed increased spreading in rheumatic fever, while recent work on the effect of salicylates gives conflicting results. Most reports agree that, *in vitro*, high concentrations are necessary to inhibit hyaluronidase; but while inhibition in therapeutic doses *in vivo* has been confirmed by some workers (Meyer, 1947; Dorfman *et al.*, 1947) it is denied by others (Swyer, 1948; Jones, 1950). Recent American literature (Symposium, 1950) favours the view that salicylates are inhibitory *in vivo*, but does not confirm the enhanced effect of spreading factor in rheumatic fever.

The well-known ability of haemolytic streptococci to form hyaluronidase (Meyer *et al.*, 1940; McClean, 1941; Crowley, 1944; Pike, 1948) has focused interest on the bacteriological relationship of this enzyme to rheumatic fever; the introduction of A.C.T.H. and cortisone, with their observed effect on connective tissue, redirects attention to the substrate and its place in the aetiology of this group of diseases. Preliminary reports indicate that in animals (Opsahl, 1949; Winter and Flataker, 1950) and in man (Shuman and Finestone, 1950) administration of these hormones causes decreased

*Based on a paper read at the Medical Research Society on July 6, 1951.