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INSULIN AS A GROWTH HORMONE

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[WITH SPECIAL PLATE]

The effect of insulin on the growth of animals deprived of the pituitary gland has not previously been reported.* The well-known obstacle has been the great sensitivity of the hypophysectomized animal to the antidiabetic hormone. Using slow-acting insulin in gradually increasing amounts and a high carbohydrate diet attractive to the animal and constantly available, we have been able to stimulate rapid growth in completely hypophysectomized rats. These animals exhibit an increase in all the main body constituents and a very definite augmentation of skeletal growth. The methods used, the details of the results, and their potential significance in physiology and medicine will be presented and briefly discussed.

Methods

The hypophysectomized rats were secured from the Hormone Assay Company. Completeness of the operation was confirmed at necropsy and no anterior pituitary tissue has thus far been found. One daily injection of protamine zinc insulin (Toronto), 40 units per ml., has been administered subcutaneously. The first lot may have contained a little hyperglycaemic factor, but this material could not be detected in subsequent ones. The composition of the diet was as follows: commercial dog food containing horse meat 892 g., white bread crumbs 345 g., whole wheat flour 396 g., glucose 80 g., and 1 quart of whole milk.

Animals in all groups ate the same diet *ad libitum*. The food intake and the weight of each rat were measured daily. Organs were removed at necropsy, weighed, and returned to the carcass, which, minus the contents of the gastro-intestinal tract, was finely minced and then homogenized in a blender. Representative aliquots were thus available for the determinations of protein, fat, and water, which were carried out by standard and regularly checked methods. The tibia test of Greenspan *et al.* (1949) was performed to evaluate skeletal growth. In our second experiment two tibiae were removed from each carcass. One was placed in neutral formalin for the above-mentioned test, and the second was fixed in formol calcium. Paraffin sections were prepared and stained with H. and E. and Mallory's connective-tissue stains.

*Preliminary notes on this work have appeared (Salter and Best, 1952, 1953).

Experimental Procedures and Results

Anticipating hypoglycaemia at many points in the 24 hours, a "pilot" experiment using two rats was performed. It was found that small amounts of protamine zinc insulin were well tolerated by the hypophysectomized animals, and that when a suitable diet was made available at all times constant attention became unnecessary. Before this goal was reached in this experiment one animal succumbed to hypoglycaemia, but the other grew rapidly, nearly doubling its weight in 20 days, and it served as a model for the many larger series of animals which have subsequently been studied. In this rat it was found that the tail had lengthened by 1 cm. during the 20 days of insulin therapy. At necropsy the thymus, kidney, and heart weights were within the normal range. The liver was heavier than normal. The testes, adrenals, and thyroid were atrophic.

Experiment A.—In this experiment 40 male rats, 14 days after hypophysectomy, were divided into three groups of

TABLE I.—*Organ Weights of Hypophysectomized Rats*

	No. in Group	Adrenals		Testes		Thymus		Kidney		Heart		Liver	
		Weight (mg.)	% Body Weight	Weight (g.)	% Body Weight	Weight (g.)	% Body Weight	Weight (g.)	% Body Weight	Weight (g.)	% Body Weight	Weight (g.)	% Body Weight
Hypophysectomized controls killed at start of experiment	10	12·6	0·011	0·53	0·47	0·235	0·206	0·72	0·63	0·34	0·30	3·61	3·16
Hypophysectomized controls; no treatment 16 days	11	12·6	0·011	0·34	0·29	0·303	0·263	0·71	0·62	0·36	0·31	3·79	3·29
Hypophysectomized rats receiving protamine zinc insulin 15 days	8	14·2	0·009	0·31	0·20	0·42	0·27	0·87	0·57	0·44	0·29	7·45	4·87

TABLE II.—*Carcass Composition of Hypophysectomized Rats*

Treatment	No. in Group	Average % Composition			Average Absolute Weight			Average in g.		Average Width (μ) of Epiphysial Cartilage
		Water	Fat	Protamine	Water	Fat	Protamine	Terminal Body Weight	Weight Change	
Hypophysectomized control skilled at start of experiment	10	67·4	10·6	20·4	76·8	12·1	23·2	114		
Hypophysectomized controls; no treatment 16 days	11	65·4	13·3	19·2	75·2	15·3	22·1	115	+4	114
Hypophysectomized rats receiving protamine zinc insulin 15 days ..	8	64·1	16·8	17·5	98·1	27·5	26·8	153	+41	186

10, 18, and 12. The 10 animals were sacrificed on the initial day to secure basal values. Each member of the second group received daily for 15 days a dose of protamine zinc insulin, which was increased whenever the previous dose failed to produce a definite increase in weight. The 12 animals in group 3 received no insulin and were sacrificed at the same time as those in group 2. The analytical results secured on the surviving animals are given in Tables I and II, and the growth curves are plotted in Fig. 1.

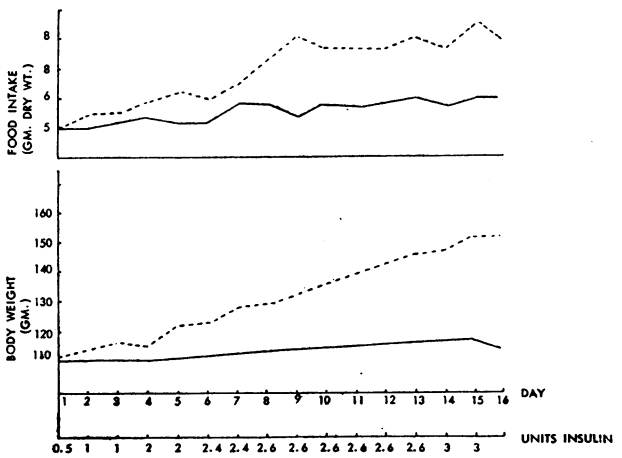

FIG. 1.—Weight changes and food intake of hypophysectomized rats. ———, controls. - - - - - -, receiving protamine zinc insulin.

In Table I the figures demonstrate that insulin has produced an absolute increase in the weights of thymus, kidney, heart, and liver. In Table II the figures show that the gain in weight of the insulin-treated animals was due to an absolute increase in the main body constituents. The results of the tibia test showed that very definite skeletal growth had occurred. Total body fat increased to a greater extent than protein and water. There was, however, a definite increase in protein. There appears to be a slight reduction in total protein in the control animals, but this may not be significant. The curves in Fig. 1 are self-explanatory.

Experiment B.—In this experiment 40 male rats, 14 days after hypophysectomy, were divided into groups of 20, 10, and 10. Each rat in group 1 received, as in Experiment A, one daily dose of protamine zinc insulin. Each rat in group 2 received a daily subcutaneous dose of growth hormone, which was regulated to produce approximately the same rate of growth as exhibited by the insulin-treated animals. Group 3 served as a control and received the same diet *ad libitum* as the other two groups. All the animals were sacrificed on the 15th day of the experiment. The analytical findings in the surviving animals are presented in Tables III and IV and the growth curves in Fig. 2.

*Maximal amounts of growth hormone were not administered. Somewhat larger doses of insulin might also have been given. In this experiment the weight gains of the two groups were matched by design.

The findings confirm those of Experiment A and indicate that under the conditions of this study the insulin-treated hypophysectomized rats stored more fat and less water than similar ones treated with G.H. The animals receiving P.Z.I. consumed more food (including protein), but their total body protein was slightly less than that of the animals treated with G.H. The insulin-treated rats retained a smaller proportion of the ingested nitrogen. The average weight gain and final weight were the same in both experimental groups.* Insulin appeared to produce a greater gain in weight of kidneys, heart, liver, and thymus. Neither hormone exerted any definite effect on the adrenal, thyroid, or testes.

Experiment C.—In this experiment 53 hypophysectomized rats were divided into groups 1, 2, and 3, consisting respectively of 14 controls, 14 treated with growth hormone, and 25 treated with protamine zinc insulin. The procedure followed was identical with that described in Experiment B. During the experimental period 8 of the insulin-treated animals died. All rats in groups 1 and 2 survived. The increase in weight of both insulin- and growth-hormone-treated animals was 42 g. in the 15 days. The dosage of both materials was approximately the same as in Experiment B (see Fig. 2). The gain in protein was 2.6 g. per animal in the insulin-treated and 4.0 in the G.H.-treated group. The increase in width of the epiphysial disk was 75 μ in the insulin-treated group and 154 μ in the G.H.-treated. In a normal rat weighing the same as the insulin-treated animal the epiphysial disk was approximately the same width. The growth hormone increased the width of the disk to almost twice that of a normal intact control rat of the same weight. Fig. 3 (Special Plate) shows sections of the epiphysial disks of treated and untreated hypophysectomized rats.

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FIG. 3A represents a section of the epiphysial disk of a hypophysectomized control rat. All zones of the epiphysial disk are very narrow. The trabecular bone is very coarse and the bone marrow is very fatty in both epiphysis and metaphysis. This section illustrates the characteristic effects of hypophysectomy on the tibia of a young rat.

FIG. 3B shows the stimulating effect of insulin administration on the skeletal growth of hypophysectomized rats. Professor W. S. Hartroft chose as a representative disk one which was roughly twice as wide as the disks of untreated hypophysectomized rats. The histological picture approximates that of a normal intact rat of the same weight (see Fig. *C*). The organization of both the epiphysial and metaphysial bones is coarse and irregular, with pronounced growth lines indicated by irregular waves and bands. The marrow of the epiphysis and metaphysis is less fatty than that of the control animals.

FIG. 3C is a representative photomicrograph made through the epiphysial disk of a normal intact male rat weighing the same as the average weight of the insulin-treated hypophysectomized animals (143 g.). The organization and arrangement within the disk are very orderly and no irregularities are apparent.

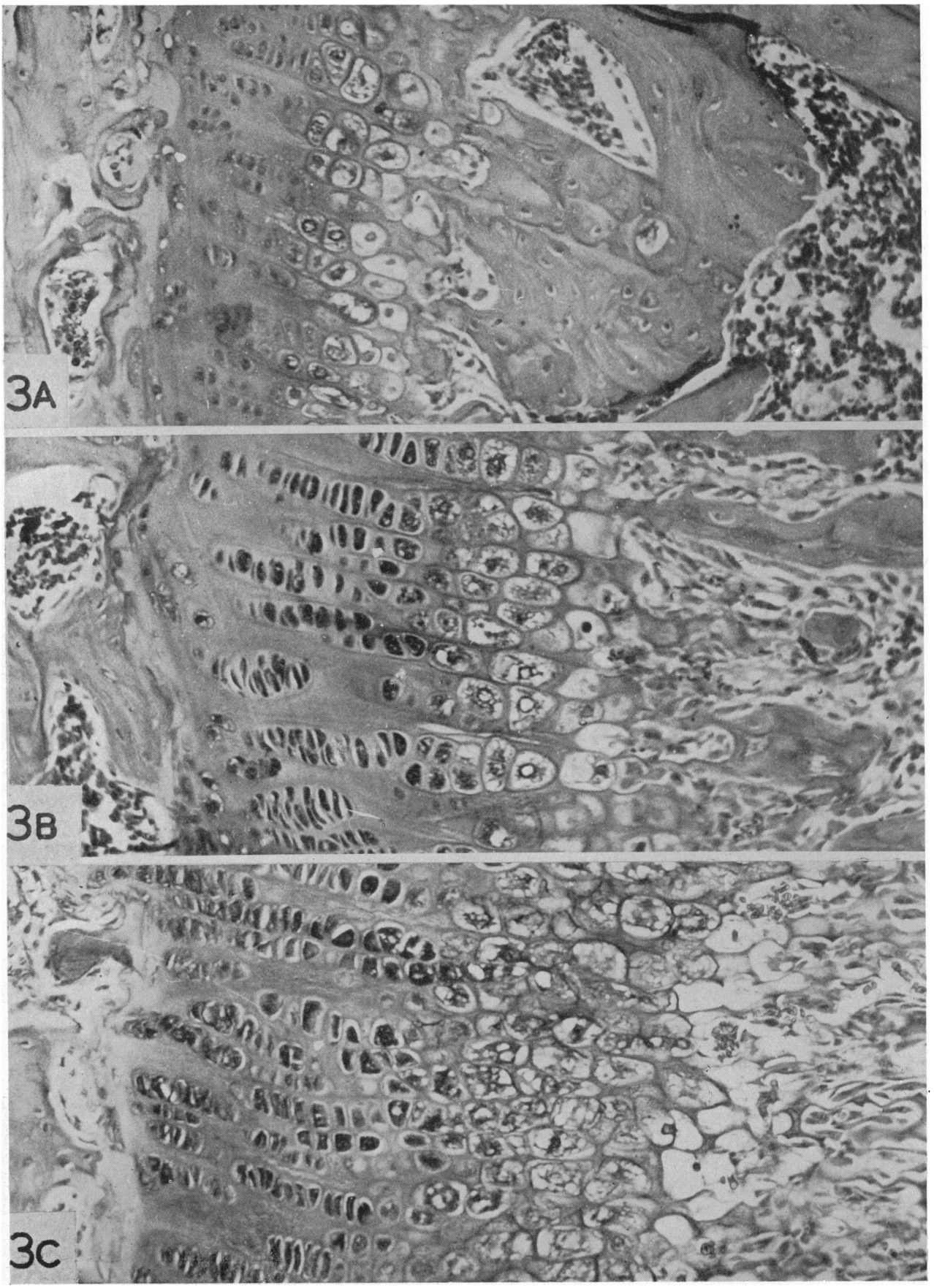
3A
3B
3C

TABLE III.—*Organ Weights of Hypophysectomized Rats*

	No. in Group	Adrenals		Testes		Thymus		Kidneys		Heart		Liver	
		Weight (mg.)	% Body Weight	Weight (g.)	% Body Weight	Weight (g.)	% Body Weight	Weight (g.)	% Body Weight	Weight (g.)	% Body Weight	Weight (g.)	% Body Weight
Hypophysectomized controls; no treatment 15 days	10	13·7	0·012	0·36	0·32	0·26	0·25	0·63	0·57	0·31	0·28	3·65	3·30
Hypophysectomized rats; growth hormone injected 14 days	10	15·7	0·011	0·33	0·23	0·37	0·26	0·76	0·54	0·37	0·26	4·65	3·30
Hypophysectomized rats; protamine zinc insulin injected 14 days	12	14·6	0·010	0·33	0·23	0·44	0·31	0·81	0·57	0·42	0·30	5·59	3·96

TABLE IV.—*Carcass Composition of Hypophysectomized Rats*

Treatment	No. in Group	Average % Composition			Average Absolute Weight			Average (g.)		Average Width (μ) of Epiphysial Cartilage
		Water	Fat	Protamine	Water	Fat	Protamine	Terminal Body Weight	Weight Change	
Hypophysectomized controls; no treatment 15 days	10	64·7	14·0	20·0	71·8	15·5	22·2	111	+4	120
Hypophysectomized rats; growth hormone injected 15 days	10	67·3	13·6	18·6	94·9	19·3	26·2	141	+34	275
Hypophysectomized rats; protamine zinc insulin injected 15 days	12	65·0	15·7	17·6	91·6	22·1	24·8	141	+33	196

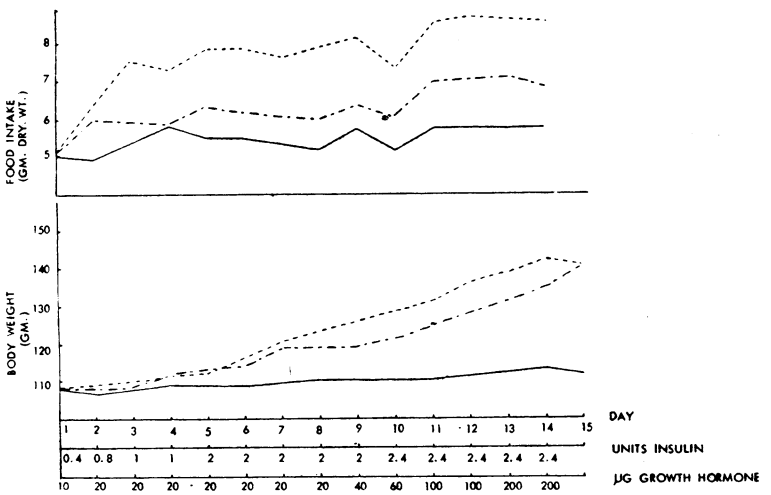

FIG. 2.—Weight changes and food intake of hypophysectomized rats. ———, controls. — - — - —, receiving growth hormone. ------, receiving protamine zinc insulin.

Discussion

It is well established that insulin plays an important part in anabolic processes, and that it reverses the series of catabolic reactions which are collectively known as diabetes mellitus. Insulin causes resumption of growth in the depancreatized animal. It increases the rate of formation of glycogen and fat and of the incorporation of amino-acids into proteins. The only catabolic effect of physiological amounts is to increase the combustion of sugar. Under physiological conditions insulin works synergistically with the somatotropic factor of the anterior pituitary and with other hormones which in the complete absence of insulin exert predominantly catabolic effects.

When the pituitary gland is removed growth may be prevented (Allen, 1916; Evans and Long, 1921). The animals remain at approximately the same weight level. The growth rate is zero. But in many species when the source of insulin is completely removed there is a rapid loss in weight. The growth rate is negative. Removal of the pituitary from these depancreatized animals may restore the growth rate to zero.

The hypophysectomized animal may develop fatal hypoglycaemia during even a short period of fasting, without receiving any exogenous insulin, and this is probably the principal reason why experiments such as we are reporting have not previously been carried out. We have used only one daily dose of P.Z.I. It is quite probable that a more continuous insulin therapy might reveal even more dramatic effects of insulin upon the growth of hypophysectomized animals.

The well-established anabolic effects of somatotropin in the normal or hypophysectomized animal do not require comment here. *The protein retention and skeletal growth are both much greater than that which we have produced with insulin alone. In the somatotropin-treated series both insulin and the pituitary growth hormone were available. Somatotropin without insulin produces no nitrogen retention or skeletal growth* (Milman *et al.*, 1951). Our experiments provide no further evidence on this latter point or on the debatable question of the liberation of insulin from the islets by the indirect or direct action of somatotropin. It is remotely possible that somatotropin, which produces a large increase in the volume of the pancreatic islets, causes a more effective liberation of insulin than we have accomplished with one daily dose of P.Z.I., but there is no *direct* evidence as yet that somatotropin causes any liberation of insulin.

It has been reported that somatotropin may cause liberation of glucagon, the pancreatic hyperglycaemic factor (Bornstein *et al.*, 1951), and, recently, that glucagon may stimulate growth, as evidenced by widening of the epiphysial disks of hypophysectomized rats (Elrick, 1953). In these latter experiments the most potent preparation of glucagon did not produce a convincing widening of the epiphysial disks. Convulsions, relieved by sugar, were frequently a late result of the injection of other preparations. These could have been produced by some residual insulin or perhaps, as suggested by the author, by the reaction of the beta cells to the hyperglycaemia. In an extension of findings by earlier investigators, Dr. O. Sirek, in our laboratory, has demonstrated that a purified growth preparation may produce no hyperglycaemia in a normal dog but a large further increase in blood sugar in the same dog after pancreatectomy. The blood-sugar changes that occur under these conditions suggest that during a period of growth-hormone administration the hypoglycaemic factor may be

liberated from the pancreas in more effective amounts than is the hyperglycaemic principle.

The clinical significance of this experimental demonstration—that is, the production of rapid growth in the "pituitary"-dwarf animal by giving insulin—merits consideration. Is a failure of insulin liberation one of the many factors which may limit growth in the human pituitary dwarf? Removal of the pituitary in animals does not cause atrophy of the islands of Langerhans, but it prevents their growth and presumably the augmented liberation of insulin which this normal growth would require and elicit. It may be argued that any factor or factors which produce general body growth will cause growth of islet tissue. This is probably true under most circumstances (Haist, 1953). It would not be expected in this present study, since the excess of insulin would lessen the need for islet activity, reduce the granule count of the beta cells, and lower the insulin content of the pancreas.

Some human "pituitary" dwarfs are very sensitive to insulin; others are not. The evidence implicating the pituitary at all is frequently most unconvincing. Our findings show that in one species the presence of low blood-sugar values and great sensitivity to insulin do not eliminate administration of insulin as one means of producing a dramatic growth effect. To what extent the hypoglycaemic effect of insulin may be modified by other hormones without interfering with its growth effect is being investigated both experimentally and clinically.* A more fundamental clinical question is whether or not a failure of growth ever occurs, apart from the frank diabetic state, in which the limiting factor is the ability of the beta cells to produce and liberate insulin. Our findings *do not* show that exogenous insulin is necessary for growth of hypophysectomized rats. Somatotropin, when endogenous insulin is available, is more effective. But a clinical condition might exist in which the pancreatic beta cells, while still capable of preventing the diabetic state at the existing reduced rate of growth and demand for insulin, are one of the factors limiting normal development. Somatotropin and insulin together might produce results where one alone would be inadequate. The clinician watches for evidence of decreased function of thyroid, adrenal, and gonads in cases of suspected pituitary dwarfism. He may attempt to stimulate these glands or to provide their products. We are suggesting that, in addition, a condition may exist in which the stimulus exerted by somatotropin or by an increased food intake may prove inadequate to provide an optimum liberation of insulin from the pancreas. Would this situation always be revealed by the appearance of diabetes? Are there other procedures which may stimulate the beta cells? Will insulin administration further decrease the response of the beta cells?

Some growth studies have been made in animals with small amounts of insulin and large amounts of somatotropin available, but many other variations of dosage of these two hormones and of insulin with other hormones will certainly yield new and interesting findings. Some investigators have confused the issue by assuming, without justification, that the alloxan-diabetic animal has *no* insulin available.

With our high-carbohydrate diets the effect of insulin on fat synthesis has been conspicuous. With very high protein diets a different picture might be obtained, but the tolerance for insulin would probably be reduced. The findings in Querido's laboratory in Leyden (personal communication) indicate that the duration of feeding periods and their spacing throughout the day may affect the composition of body tissues. The time of administration and the duration of action of the various hormones might affect appetite, the pattern of food intake, and perhaps the composition of body tissues.

*Mr. Robin Lawrence and one of us (J. S.) have found that cortisone may prevent the effect of insulin on the widening of the epiphysial disk, but the hypophysectomized animals receiving both hormones gained appreciably in weight.

As illustrated in Figs. 1 and 2, the insulin-treated hypophysectomized rats consumed more food than the controls. There is evidence that insulin-treated normal rats (Maassen, 1951) grow faster than paired-fed controls, and this is certainly clearly seen when somatotropin is the growth stimulant (Li and Evans, 1948; Young, 1952). The effect of forced feeding on the growth of hypophysectomized rats is controversial. Samuels, Reinecke, and Bauman (1943) state that force-fed hypophysectomized rats exhibit an increase in femur and body weight. Levin (1944), in repeating these experiments, reported that the increase in body weight could be totally attributed to fat deposition. It will be difficult to give much insulin to hypophysectomized rats which are not permitted to eat more than the controls. A careful comparison of force-fed and insulin-treated hypophysectomized animals with identical food intakes might provide valuable data. Great care in the interpretation of results would be necessary, since the effect of insulin, liberated physiologically by the demands of a forced food intake, might not affect growth in exactly the same way as would an identical food intake stimulated by an excess of exogenous insulin given in one or more daily doses.

Summary and Conclusions

The sensitivity to insulin of hypophysectomized animals has prevented investigation of the effects of the antidiabetic hormone on their growth. By using a slow-acting insulin in gradually increasing doses an average increase in weight of 38 g. in 15 days has been secured in completely hypophysectomized rats. The width of the epiphysial disk of the tibia in these three experiments showed, on the average, an increase of 65%. The average increase in body protein was 2.6 g. per rat. The control hypophysectomized animals which ate smaller amounts of the same diet remained essentially stationary in weight, protein content, and skeletal size. These findings therefore demonstrate that insulin may stimulate growth under these conditions and strengthen the possibility that decreased liberation of insulin may be one of the factors limiting growth in the hypophysectomized animal. The physiological and clinical significance of these findings has been briefly discussed.

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The Council of the Yugoslav Academies has commenced publication of a quarterly *Scientific Bulletin* in English, French, and German, the chief purpose of which is to give information on scientific activities in that country. Most scientific work in Yugoslavia appears only in periodicals published in the Croatian or Serbian languages, which makes it difficult for those abroad to become acquainted with current research work. For this reason alone the *Bulletin* should prove of considerable interest. It is edited by Dr. A. Peterlin, Institut de Physique J. Stephan, Ljubljana, Yugoslavia.