the greatest activity appeared at a concentration of 1% with a definite decrease in activity below and above this concentration.

Inasmuch as there were no significant differences in initial activity regardless of the concentration of medium constituents employed, and since marked differences were noted after successive washings, it may be concluded that the nutritional status of the cells, insofar investigated, affected either the stability of the enzyme system or the permeability of the cell membrane. The experiments were carried out with and without the addition of cofactors (1 mg yeast extract per tube) with no significant difference in results, indicating that coenzyme concentration was not limiting in these studies. It is important to note that both the amount of growth and the final pH varied only slightly among the various media studied.

Discussion. The results given indicate that the initial enzymic activity (aspartate deaminase system) of the bacterial cells is not influenced by the variations in nutrition studied. Upon the application of a criterion not previously employed, namely enzyme activity after successive washings, it became apparent that the nutrition during growth does affect significantly the activity of this enzyme system. Further work is necessary in order to elucidate the mechanism of these effects. Two possible explanations may, however, be offered. These are altered enzyme stability or differences in cell membrane permeability. In the latter case the effect may be due to either lowered permeability to the substrate or increased permeability resulting in loss of enzyme from the cell.

These results are significant not only because they suggest a more careful appraisal of the effects of nutrition on an enzyme system under investigation, but also because they reemphasize the fact that the enzyme constitution of a particular bacterial suspension is that portion of its potential which is selected by the nature of the growth medium, the conditions of cultivation, and the methods of harvesting and preparing the cell suspensions.

It appears to us not at all improbable that more rigid attention to these factors will lead to the discovery of enzyme systems predicted but hitherto not described, and to the stability of systems at present difficult to study.

Summary. Data are presented to show that certain variations in nutrition, while not affecting the initial activity of an enzyme system, may influence markedly the stability of this system to successive washings.

1. Gale, E. F., *Bact. Rev.*, 1943, v7, 139.

Received September 30, 1952. P.S.E.B.M., 1952, v81.

Prevention of Growth Hormone-Induced Diabetes in Hypophysectomized Dogs by Adrenocortical Steroids.* (19899)

R. C. DE BODO, M. W. SINKOFF, S. P. KIANG, AND H. DEN.

From the Department of Pharmacology, New York University College of Medicine.

In an earlier investigation it was shown that growth hormone (Armour), in dosages of 1.0 mg/kg/day intramuscularly, completely abolished the insulin hypersensitivity of hypophysectomized dogs after several days administration, but always produced a concomitant diabetes and insulin resistance(1-3). In addition growth hormone usually produced toxic effects (anorexia, vomiting, lethargy, death). If growth hormone has a truly physiological role in carbohydrate metabolism, it was postulated that 1) administered growth hormone, in the proper dosages under proper conditions, should partially or totally abolish the insulin hypersensitivity of the hypophysectomized dog without producing diabetes and 2) could then be administered indefinitely without the

* Aided by grants from the National Institutes of Health, U. S. Public Health Service, Eli Lilly and Co. and the American Cancer Society recommended by the Committee on Growth of the National Research Council.

appearance of any unphysiological effects. In an attempt to demonstrate such a physiological anti-insulin action without the production of diabetes, the dosages of the growth hormone regimens were reduced. When growth hormone was administered to hypophysectomized dogs in the smallest effective dose (0.02 mg/kg/day intramuscularly) that exerted noticeable anti-insulin effects, it was found that 50% of the dogs so treated still developed a diabetic-type of glucose tolerance(4).

Previous work had shown that either growth hormone(5,6) or C-11, 17-oxycorticosteroid (cortisone or hydrocortisone) regimens(7,8) could elevate the markedly depressed renal functions of hypophysectomized dogs only partially towards normal, but neither of these 2 hormonal factors could restore the renal functions completely to normal levels. In view of these findings, a combined adrenocortical steroid-growth hormone regimen was administered to hypophysectomized dogs to study the effect on renal functions,† and concurrently, observations were made on the changes effected in the carbohydrate metabolism of these animals. The present report is concerned with the effects of combined adrenocortical steroid-growth hormone regimens on the carbohydrate metabolism of hypophysectomized dogs.

Methods. A series of trained, unanesthetized female hypophysectomized dogs (53 to 222 days post-hypophysectomy) were used in this investigation. The technic of hypophysectomy(9),‡ after-care and feeding(3) of these animals are described elsewhere. All dogs had to exhibit a marked insulin hypersensitivity before they were chosen for this study. All experiments were done in the postabsorptive state. Insulin sensitivity was determined by the hypoglycemic response to 0.025 unit/kg insulin (Lilly)‡ administered intravenously (designated hereafter as the "test dose"); blood sugar changes were followed for 4 hours. In certain experiments a larger dose of insulin was used, 0.25 unit/kg intravenously, *i.e.*, 10× the test dose. The glucose tolerance was determined by the 4-hour blood sugar changes following a 10-minute intravenous infusion of 0.075 g/kg/min. glucose. The response to the hyperglycemic action of adrenaline was tested by a 5-minute intravenous infusion of adrenaline hydrochloride, 0.0035 mg/kg/min. The adrenocortical steroids, cortisone acetate or hydrocortisone (compound F) acetate‡ were given in daily dosages of 0.83 to 1.2 mg/kg intramuscularly in 2 divided doses (9 A.M., 5 P.M.) for periods of 17 to 38 days at which time growth hormone administration was begun, while the adrenocortical steroid regimen was continued. These dosage regimens of adrenocortical steroids, while sufficient to re-

TABLE I. Responses of Hypophysectomized (Hyphex) Dog K-44 to Test Dose of Insulin (.025 u/kg i.v.): a) in the Untreated State, b) During a Compound F Regimen Alone, and c) During a Combined Compound F-Growth Hormone (GH) Regimen Compared with That of Normal Dogs.

		Hyphex dog K-44		
Time, min.	Normal* composite	Untreated state	Cpd. F† alone	Cpd. F and GH‡
	Blood sugar in mg %			
0	78	66	64	72
	Insulin .025 u/kg intrav. at 0 time			
10	67	55	60	64
20	66	41	54	58
30	74	40	58	61
40	75	41	62	68
50	77	39	71	72
60		39	74	72
75		40	73	72
90		41	73	73
120		45	71	72
150		51	75	71
180		63	77	72
210		63	77	72
240		63	75	73

* Based on 37 insulin sensitivity tests on 18 normal dogs.
† Cpd. F, .83 mg/kg/day, given for 12 days.
‡ " " " " " " 28 " ; GH, 1 mg/kg/day, for 11 days.

† The renal function studies were done in collaboration with Drs. D. P. Earle and S. J. Farber and the findings will be reported elsewhere.

‡ We should like to express our appreciation to: Dr. A. C. Bratton, Jr., of Parke, Davis Co., for Thrombin Topical and "Penicillin S-R"; Dr. R. K. Richards of Abbott Laboratories for Pentothal Sodium and Penicillin G Procaine Aqueous; Dr. K. K. Chen of Eli Lilly and Co. for the Insulin and Duracillin; Dr. E. Alpert of Merck and Co. for "Hydrocortone Acetate" (compound F); and Drs. E. E. Hays and I. M. Bunding of Armour Laboratories for the growth hormone.

store the deranged carbohydrate metabolism of adrenalectomized dogs to normal, nevertheless concomitantly elevated their renal functions far above normal levels(10). In view of this effect, the steroid regimens were considered to be above the physiological range. The growth hormone (lot No. J21609R supplied by Armour Laboratories‡) was given in dosages of 1.0 to 1.5 mg/kg intramuscularly once daily, usually after the afternoon feeding, and continued for 12 to 31 days in conjunction with the adrenocortical steroid regimens. Thus the adrenal steroid regimens were administered for periods totalling 39 to 69 days.

Results. In these studies, all the hypophysectomized dogs were first placed on either cortisone or compound F regimens. The effects produced by these 2 hormones on the dogs' carbohydrate metabolism were similar. Table I records the various responses to the test dose of insulin, 0.025 unit/kg intravenously, of dog K-44, a typical hypophysectomized dog in this series. A compound F regimen, 0.83 mg/kg/day, abolished the insulin hypersensitivity of this dog, *i.e.*, it prevented the marked blood sugar fall seen in the untreated hypophysectomized state and accelerated the recovery of the blood sugar to the postabsorptive levels at a rate similar to that occurring in normal dogs. However, the postabsorptive blood sugar was not elevated in this case at the time that the experiment was performed, although it was frequently observed to be at normal levels during the compound F regimen. The glucose tolerance on the compound F regimen showed the following: 1) abolition of the secondary hypoglycemia which is a characteristic of the untreated hypophysectomized state, 2) no significant rise in the peak of the blood sugar at the termination of the glucose infusion and 3) no shift to the right in the decline of the blood sugar (Fig. 1); thus, a normal glucose tolerance was produced. The adrenaline response of steroid-treated dog K-44 also revealed a parallel improvement and approached the average blood sugar rise seen in normal dogs. Hence, the steroid regimen apparently restored the carbohydrate metabolism of these hypophysectomized dogs to normal (as judged by the above criteria). This was in agreement

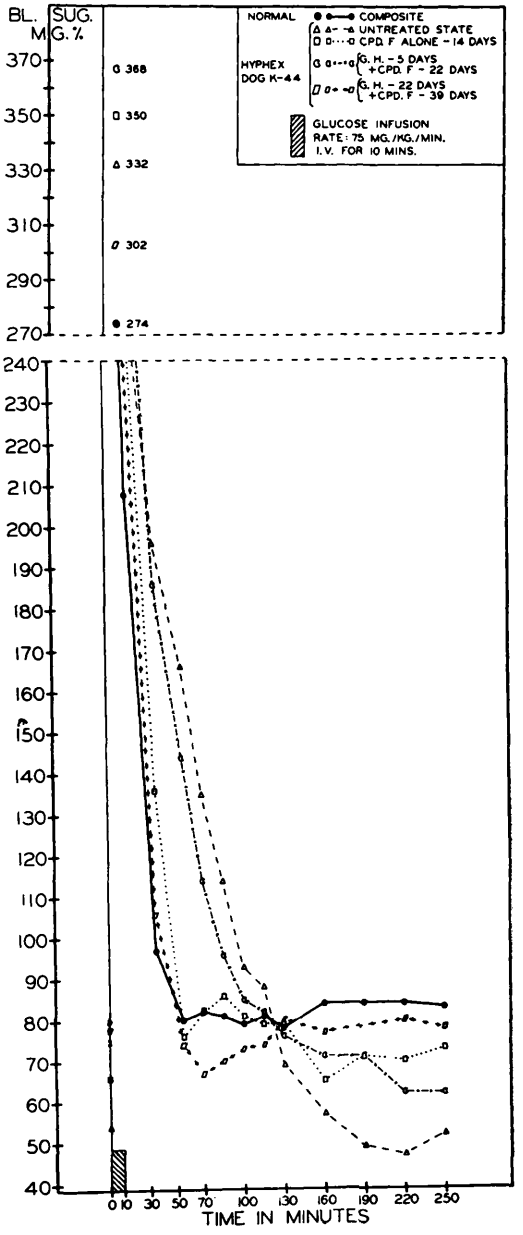

FIG. 1. Blood sugar curves produced by intrav. glucose (.75 g/kg) in a) normal dogs, and in hypophysectomized (hyphex) dog K-44, b) during the untreated state, c) during a compound F (.83 mg/kg/day) regimen alone, and d) during a combined compound F-growth hormone (1 to 1.5 mg/kg/day) regimen. Compound F given for a total of 39 days. Growth hormone Armour #J21609R, 1 mg/kg/day, begun on the 18th day of the compound F regimen and continued for 15 days. Dosage of growth hormone then raised to 1.5 mg/kg/day and given for 7 more days.

with previously published work on the effects of cortisone on the disturbed carbohydrate metabolism of hypophysectomized dogs(11).

At this stage in the course of the adrenocortical steroid regimens, *viz.*, when they had produced a normal carbohydrate metabolism in the hypophysectomized dogs, growth hormone regimens were begun in combination with the steroids and continued for as long as 31 days without the appearance of any toxic manifestations. In the case of dog K-44 a growth hormone regimen, 1.0 mg kg day intramuscularly, was begun after the animal had been on a compound F regimen for 17 days. After 11 days on such a combined hormonal regimen (the 28th day of compound F therapy), the insulin response of dog K-44 revealed no change from that seen on the steroid regimen alone (Table I). Likewise, the glucose tolerance of dog K-44 on such a combined hormonal regimen remained unaltered from that obtained on the steroid regimen alone. Fig. 1 records the glucose tolerances of dog K-44 after 5 and 22 days on a combined steroid-growth hormone regimen (the 22nd and 39th days, respectively, of the compound F regimen). Indeed, the glucose tolerance test after 22 days of growth hormone and 39 days of compound F was carried out at a time when the growth hormone dosage had already been increased from 1.0 to 1.5 mg kg day for 7 days prior to this test, and yet the glucose tolerance still remained normal. Similarly, the adrenaline response remained at normal levels on the combined hormonal regimen. In some exceptional cases, the maximum blood sugar rise in response to intravenous adrenaline was above the normal range.

Although the responses to insulin and intravenous glucose of these hypophysectomized dogs were restored to normal by the combined hormonal regimens, further tests were performed with larger doses of insulin, 0.25 unit kg intravenously (10× the test dose) in order to ascertain to what degree, if any, insulin resistance had developed in these dogs during the course of therapy. However, the animals still responded like normal dogs to this large dose of insulin. This result was in marked contrast to the results previously obtained with growth hormone regimens alone in which marked insulin resistance was produced (even to such doses as 1.5 units/kg insulin intravenously)(3).

The Armour growth hormone preparation lot No. J21609R used in the above studies was administered alone to several hypophysectomized dogs and produced complete abolition of their insulin hypersensitivity, *i.e.*, the postabsorptive blood sugar was elevated and practically no fall in the blood sugar occurred after insulin. In addition a marked tendency towards diabetes was noted on all these dogs' glucose tolerance tests and indeed, cessation of administration was necessitated in some dogs in view of the onset of severe toxic signs (anorexia and vomiting) which proceeded eventually in some cases, even to death. Thus, there was no doubt that an active and potent "diabetogenic" growth hormone preparation had been given in conjunction with the various adrenocortical steroid regimens. Reversal of the order of administration of the 2 hormonal agents was planned. In other words, growth hormone was to be given first to hypophysectomized dogs until it would have produced diabetes and insulin resistance. At that time an adrenocortical steroid regimen was to be given in conjunction with the growth hormone regimen. However, this approach was discontinued when after only 7 days administration of Armour No. J21609R, 1.0 mg/kg/day, hypophysectomized dog S-2 died. Dog S-2 was one of the animals that was used in the above studies in which the steroids were given first. Indeed, hypophysectomized dog S-2 had previously received growth hormone Armour No. J21609R, 1.0 mg/kg/day for 31 days, together with a cortisone regimen, 1.0 mg/kg/day, without any ill effects whatsoever.

Discussion. From the above results it can be seen that an adrenocortical steroid regimen (either cortisone or compound F) can restore the carbohydrate metabolism of hypophysectomized dogs to normal, as judged by their responses to insulin, intravenous glucose and adrenaline, without producing insulin resistance, diabetes, or abnormally high adrenaline responses. However, the dosage range employed was deemed in excess of physiological levels in view of the abnormally elevated renal functions of adrenalectomized dogs produced

by these same steroid dosages. Moreover, the administered steroids were augmenting the action of the hypophysectomized dogs' own adrenaline(11) and supplementing their adrenocortical steroid secretions. Nevertheless, when a growth hormone regimen was given in conjunction with the adrenal steroids, no further changes were noted in the hypophysectomized dogs' responses to insulin, intravenous glucose, and adrenaline. Thus, the normal carbohydrate metabolism produced by steroid regimens alone remained unchanged on combined adrenal steroid-growth hormone regimens. The question therefore arose, was the growth hormone preparation used in these experiments contributing at all to the pronounced anti-insulin or, more broadly speaking, blood sugar-raising effect of the adrenal steroids? As mentioned above this growth hormone preparation was an active and potent "diabetogenic" preparation inasmuch as it exerted marked anti-insulin and diabetogenic actions when administered alone to previously untreated hypophysectomized dogs. Furthermore, it was found that the renal functions of these same hypophysectomized dogs which were only partially restored towards normal levels during the steroid regimens alone, were elevated to normal levels during the combined adrenocortical steroid-growth hormone regimens. When the growth hormone regimens were discontinued, the animals thereby receiving only adrenocortical steroids, the renal functions fell from the normal levels to those previously observed during the steroid regimens alone. Thus, this growth hormone preparation exerted a marked effect(12).†

It is evident then, that the combined effect of 2 "diabetogenic" factors (the adrenocortical steroids and growth hormone) was neither a summation nor a potentiation of their individual effects. Furthermore, prolonged administrtion of growth hormone was made possible without the production of diabetes, insulin resistance, and toxic effects, all of which had occurred previously with growth hormone regimens alone(3). It would appear, therefore, that an antagonism existed between these 2 blood sugar-raising agents. The mechanism whereby this antagonism becomes operative is not known. It is known that 1) growth hormone requires a long period after injection into the living animal to exert its action, and 2) purified growth hormone is ineffective when added directly to the isolated diaphragm *in vitro,* but exerts its action in this preparation only if it was injected into the living animal many hours prior to the isolation of the diaphragm. In view of this, it is possible that growth hormone liberates another substance, or else undergoes some transformation. The adrenocortical steroids therefore may inhibit the secretion and/or action of this third substance or block the transformation of growth hormone. Although the results of these experiments using a combined hormonal regimen appear paradoxical at first, it is apparent that in the hypophysectomized dogs these regimens represented a replacement therapy of their absent growth hormone and deficient adrenal steroids. Moreover, the normal organism obviously does not have diabetes and yet has both growth hormone and the adrenal steroids.

The importance of an anterior pituitary factor, most probably growth hormone or some factor closely linked with it,§ in the regulation of carbohydrate metabolism cannot be denied. In previous observations, a comparison of the insulin responses of the adrenalectomized dog and of the hypophysectomized dog revealed a striking difference between the two(13). The adrenalectomized dog, while more sensitive to insulin than the normal dog, was shown to be far less sensitive than the hypophysectomized dog, although the latter animal still had an adrenaline secretion and some adrenocortical steroid secretion both of which were completely absent in the adrenalectomized dog. This pointed to the importance of an anterior pituitary factor as the

§ Raben and Westermeyer have claimed (PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 83) that their growth hormone preparation is nitrogen-retaining with little, if any, diabetogenic activity. These findings as yet have not been corroborated. Whether or not growth hormone or some other anterior pituitary factor is responsible for the actions in carbohydrate metabolism, it is still apparent that an anterior pituitary factor, in addition to ACTH, has a role in the regulation of normal carbohydrate metabolism.

agent responsible for the difference in insulin responses of these 2 experimental animals. The experiments employing a combined hormonal regimen therefore may provide a clue as to the balance prevailing between these 2 blood sugar-raising agents in the hormonal regulation of normal carbohydrate metabolism. It has been suggested that growth hormone, by interfering with peripheral utilization of sugar, raises the blood sugar and this eventually results in the exhaustion of the pancreas(3). To this relationship of growth hormone to the pancreas should be added an important interrelationship of growth hormone with the adrenal cortex on the basis of the above experiments.

Summary. 1. Continued administration of cortisone or hydrocortisone regimens, in dosages (0.83 to 1.2 mg kg day) sufficient to restore the carbohydrate metabolism of adrenalectomized dogs to normal, completely abolished the insulin hypersensitivity, secondary hypoglycemia of the glucose tolerance test, and adrenaline resistance of hypophysectomized dogs without the production of insulin resistance, diabetes, or abnormally high adrenaline responses. 2. Continued daily administration of a potent "diabetogenic" growth hormone (1.0 to 1.5 mg kg day) in conjunction with the cortisone or hydrocortisone regimens did not alter the carbohydrate metabolism of the hypophysectomized dogs previously observed on the steroid regimens alone, *i.e.*, it remained normal as judged by the above criteria. 3. The insulin resistance, diabetes, and toxic manifestations produced by growth hormone regimens alone were not observed during combined adrenocortical steroid-growth hormone regimens and thus, a more prolonged period of growth hormone administration was made possible. 4. It is suggested that a balance exists between growth hormone and the adrenocortical steroids in the regulation of the carbohydrate metabolism of the normal organism. This balance, in part, may express itself as an antagonism between the two either in the liberation of another factor or in the transformation of growth hormone.

1. de Bodo, R. C., Kurtz, M., Ancowitz, A., and Kiang, S. P., *Fed. Proc.*, 1950, v9, 30.
2. ———, PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 524.
3. ———, *Am. J. Physiol.*, 1950, v163, 310.
4. Paper in preparation.
5. de Bodo, R. C., Schwartz, I. L., Greenberg, J., Kurtz, M., Earle, D. P., Jr., and Farber, S. J., *Fed. Proc.*, 1951, v10, 33.
6. ———, PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 612.
7. Earle, D. P., Farber, S. J., de Bodo, R. C., and Kurtz, M., *Fed. Proc.*, 1952, v11, 39.
8. Paper in preparation.
9. de Bodo, R. C., Bloch, H. L., and Gross, I. H., *Am. J. Physiol.*, 1942, v137, 124.
10. Paper in preparation.
11. de Bodo, R. C., Kurtz, M., Sinkoff, M. W., and Kiang, S. P., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 345.
12. Paper in preparation.
13. de Bodo, R. C., Sinkoff, M. W., and Kiang, S. P., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 350.

Received October 2, 1952. P.S.E.B.M., 1952, v81.

Attempts to Demonstrate Interference Between Coxsackie and Poliomyelitis Viruses in Mice and Monkeys. (19900)

N. F. STANLEY. (Introduced by G. Dalldorf.)

From the Institute of Epidemiology and Preventive Medicine, Prince Henry Hospital, Sydney, Australia.

Using infant mice Dalldorf(1) demonstrated interference between a group B strain of Coxsackie virus and the Lansing strain of poliomyelitis as evidenced by decreased frequency and prolongation of the course of poliomyelitis, and by survival. On the other hand, Sulkin and Manire(2) reported that the inoculation of Lansing poliomyelitis virus into infant mice offered some slight protection to infection with a group A Coxsackie virus.