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Hormonal Factors Affecting Glucose 6-Phosphatase Activity

1. EFFECT OF HYPOPHYSECTOMY AND REPLACEMENT THERAPY IN THE RAT

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Cope & Marks (1934) observed that in hypophysectomized animals the liver glycogen appeared to be less responsive to glycogenolytic stimuli than that in normal animals. The present investigation was undertaken to ascertain whether in the hypophysectomized rat there is a diminution in liver glucose 6-phosphatase activity which might contribute to such a sluggishness in glycogenolysis, and further whether any diminution in glucose 6-phosphatase observed to follow removal of the pituitary gland in the rat could be prevented or cured by treatment with hormones.

METHODS

Animals. White rats of the Wistar strain, bred in the Laboratory, were used. Unless otherwise stated, they consumed a stock diet *ad lib.* to such an extent that their body weight rose by 35–40 g. each week. In some experiments pair-feeding was carried out. By this is meant that a pair of animals, one control and one experimental, chosen to be comparable in body weight, were fed the same amount of food each day. If one animal ate less than the other on any day, both were given the smaller amount the following day. In some experiments the principle of pair-feeding was extended to three or four comparable animals, the amount consumed by the animal eating least being fed to all.

Hypophysectomy was carried out by the parapharyngeal route. In any animal in which signs of incomplete

hypophysectomy had been observed, such as incomplete cessation of growth or failure of the gonads to atrophy, completeness of removal of the gland was checked *post mortem* by visual examination of the sella turcica. If necessary the results for the animal were discarded. Alloxan diabetes was induced in intact rats by the intraperitoneal injection of 40 mg. of alloxan/kg. body wt. Only rats which exhibited frank glycosuria were used in the experiments. Some of the alloxan-diabetic rats were subsequently hypophysectomized.

Hormone preparations. Growth hormone was prepared in the Laboratory by a method essentially that of Wilhelmi, Fishman & Russell (1948). For injection it was dissolved in 0.9% NaCl soln. at a concentration of 5 mg./ml., and 0.2 ml. of this solution (1 mg. of hormone) was administered daily by the subcutaneous route to rats under treatment. Cortisol, suspended in 0.9% NaCl soln. at a concentration of 5 mg./ml., was administered subcutaneously at a rate of 1 mg./rat/day. Sodium-L-thyroxine was dissolved in 0.9% NaCl soln. by the addition of NaOH to give a concentration of 0.5 mg. of thyroxine/ml. Of this solution 0.2 ml. (equivalent to 0.1 mg.) was subcutaneously administered each day to a rat under treatment with this hormone.

Estimation of glucose 6-phosphatase. Glucose 6-phosphatase activity was measured in a tissue homogenate. The rat was stunned, decapitated and bled, and the needed organ removed and chilled in ice. A weighed sample of the organ (200–400 mg.) was homogenized in ice-cold 0.02M-acetate-borate-cacodylate buffer, pH 6.5 (Plumel, 1948; de Duve, Berthet, Hers & Dupret, 1949), in a Potter-Elvehjem glass homogenizer. Enzyme activity was determined by the method of de Duve *et al.* (1949) but with a final substrate concentration of 0.04M. The total volume of 0.6 ml. used

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contained material from approx. 5 mg. of tissue. Phosphorus was estimated by the method of Fiske & Subbarow (1925). In agreement with Hers & de Duve (1950) significant hydrolysis of glucose 6-phosphate between pH 6 and pH 7 was found only with homogenates of liver, kidney and small intestine. The preparations from intestine were activated by Mg^{2+} ions and were optimally active at pH 9–10 so we have confined our recorded observations to liver and kidney preparations at pH 6.5.

The results of the estimation of glucose 6-phosphatase activity were expressed in two different ways. The first is in terms of mg. of P released/g. wet wt. of tissue/10 min. For convenience, results expressed in this way are referred to as 'enzyme concentration', without prejudice to the question of whether, when changes occurred, the amount of protein enzyme had varied or whether the activity of the enzyme/mg. of enzyme protein had altered. The second way in which results have been expressed is in terms of mg. of P calculated to be released by the tissues of the whole of an organ in 10 min., reduced to a figure for each 100 g. body wt. For convenience this figure is referred to as 'total activity/100 g. body wt.' for the organ in question. Where changes in glucose 6-phosphatase activity are referred to, without specific qualifications, the changes in values expressed in both ways are being considered. Both of these methods have disadvantages but there exists no simple single solution to the problem of the terms in which the enzyme content of tissues are best given. If the activity is expressed in terms of activity/g. wet wt. of tissue a fall in the amount of any of the major constituents, and particularly that of glycogen and water, could result in an apparent increase in enzyme activity, even though no rise in the total amount of enzyme in the tissue occurred. Similar considerations apply to expression in terms of activity/g. dry wt. of tissue, to activity/mg. of N and to activity/mg. of deoxyribonucleic acid P. Nevertheless results expressed in terms of total activity per organ should be uninfluenced by changes in the composition of the organ, and we have adopted the procedure of expressing total enzyme activity in an organ/100 g. body wt. to allow for both the changes in weight of the organ and the alterations in weight of the animal.

Blood sugar was estimated by the method of Somogyi (1952). Glycogen was determined by the method of Good, Kramer & Somogyi (1933), the sugar being estimated by the method of Somogyi (1952).

RESULTS

Effect of hypophysectomy on glucose 6-phosphatase activity

Liver. In control experiments (Fig. 1) the relationship between body weight and glucose 6-phosphatase activity of liver tissue was assessed for the intact growing rats of our colony. The changes in enzyme concentration in liver tissue are less striking than those in total activity/100 g. body wt. (Fig. 1). Both values rise as body weight increases from 50 to 100 g., and then decline, the difference in total activity/100 g. body wt. being substantial and highly significant ($P < 0.01$) between 100 and 350 g. rats.

Starvation for 24 hr. caused a substantial rise in glucose 6-phosphatase concentration in the livers of both the young and older rats (Fig. 1), the difference between the average values for the old and young animals being approximately the same after starvation as it was before.

When the pituitary gland was removed from the rat the glucose 6-phosphatase activity of the liver fell quite rapidly for about 2 weeks after the operation, and then more slowly or not at all (Fig. 2). The results given in Fig. 2 for enzyme

Fig. 1. Effect of age on the glucose 6-phosphatase activity of the liver of the normal (non-starving) rat. The lengths of the vertical lines on the points of the curves plotted indicate the standard errors of the mean values illustrated. —, Activity/g. of liver; - - -, total activity/100 g. body wt. Circles indicate the enzyme activities in the liver after starvation of rats of different ages for 24 hr.: ●, total activity/100 g. body wt.; ○, activity/g. of liver.

Fig. 2. Effect of hypophysectomy on the glucose 6-phosphatase concentration (mg. of P released/g. of wet tissue/10 min.) in the livers of young and of older rats. The upper curve gives the results for rats of 100–150 g. body wt. before hypophysectomy, and the lower one is for rats of 250–350 g. before hypophysectomy. The lengths of the vertical lines on the points of the curves plotted indicate the standard errors of the mean values illustrated.

concentration are similar to those, not illustrated, for total activity/100 g. body wt. and indicate that, despite the different initial values, the rate of fall is similar in older and in young rats.

In order to ascertain if the fall in activity after hypophysectomy resulted from loss of an activator of the enzyme, or possibly from the accumulation of a substance which depressed the activity of the enzyme, some experiments were carried out with mixtures of homogenates of liver tissue from normal and from hypophysectomized rats. No difference between the expected activity of the mixture and that actually observed was found.

Since the hypophysectomized rat eats less than a normal one, and starvation of an intact rat leads to a rise in liver glucose 6-phosphatase activity (Ashmore, Hastings, Nesbitt & Renold, 1956), it was of interest to compare the enzyme activity in the livers of intact rats pair-fed with hypophysectomized ones, and of normal rats fed *ad lib.* with hypophysectomized animals. The results (Table 1) show that pair-feeding greatly emphasizes the difference in enzyme concentration between the livers of normal and of hypophysectomized rats but has less influence on comparisons made on the basis of total activity/100 g. body weight.

When the hypophysectomized rat was starved for 24 hr. a small but highly significant rise ($P < 0.01$) in liver glucose 6-phosphatase concentration was seen (Table 2), though this was less than that seen for the starving intact rat (Ashmore, Hastings & Nesbitt, 1954; Harper, 1959). The rise

in total activity/100 g. original body wt. observed in these experiments (Table 2) was not significant ($P > 0.1$). The feeding of protein (casein) *ad lib.* to the starving hypophysectomized rat (Table 2) led to a rise in the glucose 6-phosphatase activity of the liver that was just significant ($P 0.05$), despite the fact that the feeding of casein to the normal rat causes a highly significant rise in the total enzyme activity in the liver (Harper, 1959).

Kidney. The glucose 6-phosphatase activity of the kidney of the hypophysectomized rat is not significantly ($P > 0.1$) lower than that of the normal rat, though the mean values for hypophysectomized rats were a little below those for control intact rats. With normal rats typical mean values for enzyme concentration, expressed as mg. of P/g. of tissue/10 min., for liver and kidney were 4.84 ± 0.2 and 4.54 ± 0.3 respectively, whereas with comparable hypophysectomized rats the corresponding values were 2.80 ± 0.3 and 4.06 ± 0.3 . In view of these findings no further investigations were carried out of hormonal effects on kidney glucose 6-phosphatase activity.

Influence of treatment with hormones on the liver glucose 6-phosphatase activity of the hypophysectomized rat

Growth hormone. Since treatment with growth hormone raises the food intake of the hypophysectomized rat, experiments both with paired-feeding and with feeding *ad lib.* were carried out. These experiments were performed in conjunction with those on cortisol (see below) and in general the food intake of hypophysectomized control rats and of hypophysectomized growth-hormone-treated rats was restricted to that of cortisol-treated hypophysectomized rats, the food intake of the last group being lowest. With feeding *ad lib.*, treatment with growth hormone for 2 weeks had little influence on the liver glucose 6-phosphatase activity of the hypophysectomized rat (Table 3). When the food intake of the growth-hormone-treated hypophysectomized rat was the same as that of the hypophysectomized rat, the total enzyme activity/100 g. body wt. was similar in the two groups ($P > 0.1$), but the enzyme concentration in the livers of growth-hormone-treated hypophysectomized rats was significantly greater ($P < 0.05$) than that in untreated hypophysectomized animals (Table 3).

Cortisol. When food intake was uncontrolled the administration of cortisol for 2 weeks to the hypophysectomized rat significantly raised both the enzyme concentration in the liver and total activity/100 g. body wt. ($P < 0.05$) above the corresponding values for hypophysectomized rats (Table 3). When these groups were pair-fed the food intake of the cortisol-treated hypophysectomized rats was lowest, so that these animals were

Table 1. Comparison of liver glucose 6-phosphatase activity of hypophysectomized rats with that of intact controls fed *ad libitum* or pair-fed

Condition of animal	Liver glucose 6-phosphatase activity (mean $\pm$ S.E.M.)	
	(mg. of P/g. of liver)	(mg. of P/100 g. body wt.)
Control (hypophysectomized)	2.9 ± 0.5	8.8 ± 1.2
Normal: pair-fed	8.0 ± 0.7	26.7 ± 1.7
Normal: fed <i>ad lib.</i>	5.2 ± 0.6	22.8 ± 1.6

Table 2. Effects of starvation and of feeding protein alone after starvation on the liver glucose 6-phosphatase activity of hypophysectomized rats

Condition of animal	Liver glucose 6-phosphatase activity (mean $\pm$ S.E.M.)	
	(mg. of P/g. of liver)	(mg. of P/100 g. body wt.)
Control (hypophysectomized)	2.2 ± 0.2	6.7 ± 0.5
Starved 24 hr.	3.6 ± 0.2	9.4 ± 0.4
Starved 24 hr. and then fed casein <i>ad lib.</i> for 24 hr.	3.8 ± 0.1	11.8 ± 0.8

in effect eating *ad lib.* in both experiments. This is the reason for the fact that the values for glucose 6-phosphatase activity for these animals are similar whether or not they were pair-fed. On the other hand, with pair-feeding control the other groups suffered some measure of food restriction and the values for the hypophysectomized control rats (Table 3) were higher, resembling those observed after starvation for 24 hr. (Table 2). Under these conditions there was no evidence of an effect of cortisol on glucose 6-phosphatase concentration, and total enzyme activity/100 g. body wt. was also not significantly different ($P > 0.1$) from that of the control animals (Table 3).

Growth hormone plus cortisol. When both of these hormones were given to the hypophysectomized rat for 2 weeks with feeding *ad lib.* (Table 3) both the enzyme concentration in the liver and the total enzyme activity/100 g. body wt. rose in a highly significant manner ($P < 0.01$). With pair-feeding control the total enzyme activity/100 g. body wt. was not significantly different ($P > 0.1$) from that of

hypophysectomized control rats (Table 3), though the concentration of enzyme was significantly increased ($P < 0.01$).

Thyroxine plus cortisol. When both thyroxine and cortisol were administered to the hypophysectomized rat for 4 days the glucose 6-phosphatase activity in the liver was highly significantly increased ($P < 0.01$), the result being greater than would be expected if the effects of the two hormones were only additive (Table 4). After a single injection of both hormones, the liver glucose 6-phosphatase activity was no greater than it was when thyroxine only was administered (Table 5) although, as before, 4 days of treatment led to a greater response to treatment with both hormones than would be expected from their separate effects (Table 5). In another experiment in which thyroxine and cortisol were given intravenously no significant change occurred in the liver glucose 6-phosphatase activity during the succeeding 4 hr.

Thyroxine plus cortisol plus growth hormone. When growth hormone was given simultaneously with

Table 3. *Effect of treatment with growth hormone and cortisol on liver glucose 6-phosphatase activity of hypophysectomized rats*

(a) Experiments with animals eating *ad lib.*; (b) experiments with animals pair-fed.

Treatment of animal	Weight change (g./2 weeks)		Liver glucose 6-phosphatase activity (mean $\pm$ S.E.M.)			
			(mg. of P/g. of liver)		(mg. of P/100 g. body wt.)	
	(a)	(b)	(a)	(b)	(a)	(b)
None	6	-7	2.5 $\pm$ 0.1	4.2 $\pm$ 0.3	8.8 $\pm$ 0.3	14.0 $\pm$ 1.0
Cortisol	-26	-21	3.9 $\pm$ 0.5	3.4 $\pm$ 0.4	15.1 $\pm$ 2.5	13.7 $\pm$ 1.8
Growth hormone	39	+14	2.7 $\pm$ 0.1	6.3 $\pm$ 0.6	9.5 $\pm$ 0.7	16.7 $\pm$ 1.8
Cortisol and growth hormone	25	-6	3.6 $\pm$ 0.2	6.6 $\pm$ 0.5	15.5 $\pm$ 0.8	17.7 $\pm$ 1.7

Table 4. *Effect of daily treatment for 4 days with various hormones on the liver glucose 6-phosphatase activity of hypophysectomized rats fed ad lib.*

Treatment	Liver glucose 6-phosphatase activity (mean $\pm$ S.E.M.)	
	(mg. of P/g. of liver)	(mg. of P/100 g. body wt.)
None	2.1 $\pm$ 0.1	7.2 $\pm$ 0.7
Cortisol	3.2 $\pm$ 0.2	11.9 $\pm$ 1.0
Thyroxine	4.6 $\pm$ 0.3	14.5 $\pm$ 0.9
Cortisol and thyroxine	8.8 $\pm$ 0.6	24.8 $\pm$ 1.4
Cortisol and thyroxine and growth hormone	8.6 $\pm$ 0.6	27.7 $\pm$ 1.5

Table 5. *Effect of administration of cortisol and of thyroxine on the liver glucose 6-phosphatase activity of hypophysectomized rats*

Period of treatment	Liver glucose 6-phosphatase activity (mean $\pm$ S.E.M.)			
	24 hr.		4 days	
	(mg. of P/g. of liver)	(mg. of P/100 g. body wt.)	(mg. of P/g. of liver)	(mg. of P/100 g. body wt.)
Treatment of animal				
None	2.1 $\pm$ 0.1	8.1 $\pm$ 1.0	—	—
Cortisol	2.6 $\pm$ 0.1	9.6 $\pm$ 0.2	—	—
Thyroxine	3.7 $\pm$ 0.5	12.0 $\pm$ 1.5	4.1 $\pm$ 0.6	14.0 $\pm$ 0.8
Cortisol and thyroxine	3.6 $\pm$ 0.4	12.5 $\pm$ 1.4	7.9 $\pm$ 0.5	25.3 $\pm$ 2.0

Table 6. *Liver glucose 6-phosphatase activity of rats treated in various ways*

Treatment of animal	Liver glucose 6-phosphatase activity (mean $\pm$ S.E.M.)	
	(mg. of P/g. of liver)	(mg. of P/100 g. body wt.)
1. None	3.8 $\pm$ 0.2	13.9 $\pm$ 1.4
2. Hypophysectomized	2.6 $\pm$ 0.2	8.8 $\pm$ 0.6
3. Alloxan-diabetic	8.4 $\pm$ 0.6	32.3 $\pm$ 2.7
4. Hypophysectomized, alloxan-diabetic	2.7 $\pm$ 0.4	7.8 $\pm$ 0.8
5. Hypophysectomized, alloxan-diabetic, with growth hormone and cortisol	5.7 $\pm$ 0.2	22.6 $\pm$ 2.5

cortisol and thyroxine no greater effect on liver glucose 6-phosphatase activity was seen than with thyroxine and cortisol without growth hormone (Table 4).

Influence of alloxan diabetes on glucose 6-phosphatase in relation to the pituitary gland

The induction of alloxan diabetes in the normal rat causes a substantial rise in the liver glucose 6-phosphatase activity (Ashmore *et al.* 1954; Langdon & Weakley, 1955). The results in Table 6 show that such an effect of alloxan diabetes is not seen in hypophysectomized rats. When the alloxan-diabetic hypophysectomized rat was treated with both growth hormone and cortisol a highly significant ($P < 0.01$) increase in liver glucose 6-phosphatase activity was found (Table 6).

Miscellaneous effects of hormone treatment of the hypophysectomized rat

In the experiments involving either a single treatment with hormones or daily treatment for 4 days, estimations of liver glycogen content and of blood sugar were carried out. No significant alterations from control values were found 24 hr. after a single treatment with cortisol and thyroxine either separately or together. After 4 days of treatment with thyroxine alone the blood sugar had fallen from 98 to 65 mg./100 ml., and the liver glycogen content had fallen from 1.5 to 0.3%. When both thyroxine and cortisol were given together, the blood-sugar value was the same as that with thyroxine alone (65 mg./100 ml.) and liver glycogen rose to a value of 2.2%.

DISCUSSION

The diminution in liver glucose 6-phosphatase activity we have observed to follow hypophysectomy in the rat was reported in a preliminary communication by one of us (Harper, 1956). This finding is not in agreement with the observations of Ashmore *et al.* (1956), who used large rats a few days after they had been hypophysectomized (conditions that would probably result in only small differences, cf. Fig. 2), but is in agreement with those of Reid, O'Neal & Lewin (1956).

The extent of the fall in liver glucose 6-phosphatase after hypophysectomy in the rat appears not to depend on the initial enzyme activity in the liver. In intact rats of 200 g. or more the enzyme activity falls as the animal grows older (Fig. 1), so that in such heavier animals the effect of hypophysectomy is less striking than it is in animals of less than 200 g. body wt. It is possible that the fall in enzyme activity with time, seen with intact rats of about 200 g. and upwards, is associated with a decline in pituitary activity, but no direct evidence for this view has been obtained.

The fact that starvation or food restriction causes a rise in the liver-enzyme activity in the normal rat, and a smaller one in the liver of the hypophysectomized rat (Table 2), complicates the interpretation of the results of experiments involving pair-feeding. A comparison of the results (Table 3) in feeding *ad lib.* and in pair-feeding suggests that the rise of liver-enzyme activity in response to growth hormone, or growth hormone plus cortisol, is associated with the restriction in food intake. The response per gram of liver was roughly proportional to the extent of the food restriction. Reid *et al.* (1956) also observed that there was a rise in the liver glucose 6-phosphatase activity of hypophysectomized rats treated with growth hormone and pair-fed with untreated hypophysectomized rats. Langdon & Weakley (1955) found no effect of treatment with growth hormone on the glucose 6-phosphatase activity of the liver in intact rats. It is possible that the rise in liver-enzyme activity brought about by starvation or food restriction is related to the increased rate of gluconeogenesis and may involve the secretory activity of either or both the adrenal cortex and the anterior pituitary gland.

Our most striking findings have been with thyroxine and with thyroxine plus cortisol (Table 5). Maley & Lardy (1955) observed that the administration of thyroxine to intact rats induced a rise in liver glucose 6-phosphatase activity, and Weber, Allard, de Lamirande & Cantero (1956) found that liver glucose 6-phosphatase activity is substantially increased in intact rats by the administration of cortisone. Ashmore *et al.* (1956)

found no diminution in concentration of the enzyme in the liver after adrenalectomy in the rat. Nevertheless, there was a fall in total activity in that organ which could be prevented by treatment with cortisol. In general our results for the effects of hypophysectomy in the rat, and the influence of hormone treatment of the hypophysectomized rat, are in agreement with what might be expected from the results with intact animals. The very high enzyme activity of liver tissue from hypophysectomized rats treated with both cortisol and thyroxine is not unexpected in view of the comparable response in the intact rat to treatment with these substances.

The fact that treatment with growth hormone, together with cortisol raises the low value for liver glucose 6-phosphatase activity seen in the hypophysectomized alloxan-diabetic rat (Table 4) does not justify the assumption that such an effect is independent of the availability of insulin in the animal, since the pancreas of the alloxan-diabetic rat has not necessarily lost all of its insulin-secretory tissue.

Ashmore *et al.* (1956) found no correlation between blood sugar and liver glucose 6-phosphatase activity, and have suggested that a high liver glucose 6-phosphatase activity might be associated with a low liver-glycogen content. However, our results with hypophysectomized rats indicate that the low liver-enzyme activity observed in this condition is associated with a low liver-glycogen content, whereas Weber *et al.* (1956) found that when rats are treated with cortisone high liver-glycogen values are observed to co-exist with high values for liver glucose 6-phosphatase activity. The effects of starvation, of treatment with thyroxine and of treatment with cortisol in raising liver glucose 6-phosphatase activity, and of hypophysectomy in inducing a fall, suggest the possibility that a rise in enzyme activity is associated with a rise in protein catabolism, and conversely. The effect of growth hormone in the pair-fed hypophysectomized rat would be compatible with such an idea since under such conditions protein may be catabolized in the liver for utilization in the muscles.

Our results suggest that the sluggishness of hepatic glycogenolysis observed by Cope & Marks (1934) may be associated with a depression in liver glucose 6-phosphatase activity. Our findings also suggest that thyroxine, cortisol and possibly growth hormone may all be involved in the influence of the anterior pituitary gland on such a process.

SUMMARY

1. Hypophysectomy of the rat is followed by a substantial fall in the liver glucose 6-phosphatase

activity but no significant change in kidney glucose 6-phosphatase activity.

2. The liver glucose 6-phosphatase activity of the hypophysectomized rat rises as the result of starvation for 24 hr. but the rise is much less than that observed in a similarly treated intact rat.

3. The administration of ox-pituitary growth hormone to a hypophysectomized rat eating *ad lib.* does not change the total glucose 6-phosphatase activity in the liver, but if the treated hypophysectomized rat is pair-fed with an untreated one, the liver glucose 6-phosphatase activity rises.

4. The administration of either cortisol or thyroxine to the hypophysectomized rat is followed by a rise in liver glucose 6-phosphatase activity, the rise with thyroxine being detectable 24 hr. after one injection of the hormone. The administration of cortisol and thyroxine together induced a substantial increase in the liver glucose 6-phosphatase activity, bringing this value above that seen in the intact rat.

5. No relationship was observed to exist between liver glucose 6-phosphatase activity and liver-glycogen content or blood-sugar level.

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