Studies on the Effect of Anterior-Pituitary Growth Hormone on Oxidative Phosphorylation in Rat-Liver Mitochondria

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Injection of anterior-pituitary growth hormone into the intact rat causes changes of metabolism leading to a greater oxidation of fat (Young, 1945; Greenbaum & McLean, 1953) and a sparing of protein (Young, 1945; Li, Simpson & Evans, 1948). The mechanism whereby these changes are effected is unknown. The diverse nature of the metabolic changes induced by the action of the hormone make it seem unlikely that they could all arise as the result of the modification of a single specific enzyme and it appeared more profitable to investigate the possibility that the effects are mediated by some primary change of a more general character.

The change could involve (i) an effect on the permeability of the cell wall itself [as has been suggested by Kipnis & Cori (1957) for the action of insulin] or on the membrane of the subcellular structures [as has been suggested by Tapley & Cooper (1956) for the action of thyroxine], or (ii) the action could be on an ubiquitous system such as oxidative phosphorylation which could modify energy relationships within the cell. Park, Meriwether & Park (1958) have suggested that thyroxine acts directly on the enzyme system carrying out oxidative phosphorylation.

In the experiments reported here it has been shown that growth hormone uncouples phosphorylation when D(-)- β -hydroxybutyrate was oxidized by rat-liver mitochondria. (Throughout this paper the lowered efficiency of oxidative phosphorylation is referred to as an 'uncoupling' for the sake of brevity, although it is realized that the effect described here is not that usually implied by the use of this term. The efficiency here seems to be lowered not by any reduction in the rate of phosphorylation but by the appearance of additional oxidation.) It has also been shown that growth hormone in very low concentration causes striking changes in mitochondrial permeability.

METHODS

Animals. Female hooded Norwegian rats were used when their body weight had reached a plateau value of 180–200 g, i.e. at about 10 weeks old.

Hormones. The growth-hormone preparation used in the bulk of these experiments was a twice-recrystallized

hormone prepared from ox-pituitary glands by the method of Wilhelmi, Fishman & Russell (1948). In addition two commercial preparations [Armour Somar A, a gift from the Endocrine Study Section of the National Institutes of Health, U.S.A., prepared by the same method, and a preparation, Batch no. 5609:2, a gift from Ferring AB, Malmö, made by the method of Raben & Westermeyer (1951)] were also used in some of the experiments on mitochondrial permeability. Other hormones used were insulin, adrenocorticotrophic hormone and prolactin (all gifts from the Biological Standards Division of the Medical Research Council). For the experiments on oxidative phosphorylation the growth hormone was dissolved in 0.9% NaCl soln. (2 mg./ml.) and 1 mg. was injected intraperitoneally 4 hr. before the animal was killed.

Reagents. Adenosine di- and tri-phosphate (ADP, ATP), oxidized and reduced diphosphopyridine nucleotide (DPN, DPNH) and triphosphopyridine nucleotide (TPN) were commercial preparations from the Sigma Chemical Co., Mo., U.S.A. Hexokinase was prepared by the method of Berger, Slein, Colowick & Cori (1945) from a yeast autolysate prepared by the method of Bailey & Webb (1948). It was purified as far as stage 3 of the Berger *et al.* (1945) procedure. Glucose 6-phosphate dehydrogenase was prepared by the method of Kornberg (1950). The method for preparing the (+) and (-) isomers of β -hydroxybutyrate was that of Lehninger & Greville (1953), which is based on an unpublished method by Dr H. T. Clarke.

Preparation of mitochondria and of 'digitonin particles'. Liver mitochondria were prepared as described by Schneider (1948) in 0.25 M-sucrose containing 10 μ M-ethylenediaminetetra-acetic acid (EDTA). The final mitochondrial pellet was washed once with 0.25 M-sucrose and then suspended in 0.25 M-sucrose to give an 8% suspension (i.e. the mitochondria from 1 g. of liver suspended in 12.5 ml. of sucrose). For experiments on mitochondrial permeability the mitochondrial suspension was prepared in 0.44 M-sucrose as described by Tapley (1956).

Digitonin particles were prepared essentially according to the method described by Devlin & Lehninger (1956). With a 1:10 homogenate of three rat livers, nuclei and cellular debris were removed (two centrifugings at 900 g for 10 min.) and the mitochondria were collected by centrifuging at 9000 g for 10 min. The mitochondrial pellet was twice washed and re-sedimented in 0.25 M-sucrose. The final mitochondrial pellet was suspended in 2.25 ml. of 0.05 M-sucrose, and 1.5 ml. of an ice-cold 2% solution of recrystallized digitonin in 0.05 M-sucrose was added over a period of 5 min. with continuous stirring. The suspension was allowed to stand at 0° for 20 min. and then diluted to 13 ml. with 0.25 M-sucrose. The suspension was then centrifuged at 40 000 g for 20 min. and the supernatant from this

operation collected and centrifuged again at 100 000 *g* for 30 min. The resulting pellet was resuspended in water (1 ml. $\equiv$ 4 g. of liver) and homogenized. Particles prepared in this manner (suggested in a personal communication from Professor A. L. Lehninger) differed from those prepared according to Cooper & Lehninger (1956) in maintaining a constant rate of phosphorylation for only about 10 min. instead of 15 min. and in being unaffected by Mg^{2+} ions at a concentration of 15 mM, whereas this concentration of Mg^{2+} ions actively uncoupled oxidative phosphorylation in the Cooper & Lehninger (1956) preparation.

Measurement of oxidative phosphorylation. For the measurement of oxidative phosphorylation with β -hydroxybutyrate, the phosphate esterified was trapped as glucose 6-phosphate, which was subsequently estimated by measuring the TPNH formed during the oxidation of the glucose 6-phosphate by glucose 6-phosphate dehydrogenase.

The rate of oxidation was measured by the rate of formation of acetoacetate since this was the only product of the oxidation when L-, D- or DL- β -hydroxybutyrate was used (see below).

The incubation mixture contained: 0.3 ml. of 0.3 M-glucose; 0.1 ml. of 0.04 M-ADP (sodium salt); 0.1 ml. of 0.3 mM-cytochrome *c*; 0.5 ml. of 0.1 M-sodium phosphate buffer, pH 7.4; 0.05 ml. of hexokinase solution (50 units of hexokinase activity); 0.1 ml. of 0.2 M- $MgSO_4$; 0.05 ml. of 1.5 M-KCl; 0.1 ml. of 0.1 M-EDTA (sodium salt); 0.2 ml. of bovine-plasma albumin (containing 7.5 mg. of albumin/ml.) and 0.3 ml. of 0.1 M-D-, L- or DL- β -hydroxybutyric acid. The mixture was equilibrated at 27° for 5 min., after which 1 ml. of the 8% mitochondrial suspension, immediately followed by 0.2 ml. of 0.02 M-NaF, was added and the whole was incubated for 5 min. At the end of this time 2 ml. was removed and pipetted into 1 ml. of ice-cold 9% perchloric acid. Controls, containing no substrate, were similarly treated. The precipitated protein was removed and the excess of perchloric acid then precipitated as the potassium salt. The final supernatant was analysed for acetoacetate and glucose 6-phosphate.

Acetoacetate was measured by the method of Greenberg & Lester (1944). Glucose 6-phosphate was measured in the following system: 0.3 ml. of the sample containing glucose 6-phosphate; 0.6 ml. of 0.25 M-glycylglycine buffer, pH 7.6; 0.6 ml. of 0.1 M- $MgCl_2$; 1.1 ml. of water; 0.2 ml. of TPN solution containing 2 μ moles; 0.2 ml. of glucose 6-phosphate dehydrogenase (10 mg./ml. of 0.1% albumin in 0.01 M-glycylglycine). The preparation of glucose 6-phosphate dehydrogenase used was virtually free of 6-phosphogluconate-dehydrogenase activity. The TPN was added last and the change in extinction at 340 *m* μ was recorded 10 min. after the addition of the TPN.

Measurement of mitochondrial permeability. The effect of hormones and proteins on mitochondrial permeability was determined by adding 0.05 ml. of a 50% mitochondrial suspension in 0.44 M-sucrose to 2.95 ml. of a sucrose solution containing the protein or hormone in a cuvette. The concentration of sucrose in the cuvette was so adjusted that the final concentration of the 3 ml. of solution was 0.25 M. At values above 0.25 M-sucrose swelling is inhibited (Lehninger, 1959).

An initial spectrophotometric reading of about 0.5–0.6 at a wavelength of 520 *m* μ was obtained and the changes in extinction were recorded over 25 min. The cuvette always included 0.1 ml. of 1.5 M-tris buffer (pH 7.4), which

effectively precluded any change of pH. In experiments involving comparison of the activity of various hormones in producing an increased permeability of the mitochondria, the activity was estimated from the change in extinction between the fifth and fifteenth minutes.

Measurement of the pyridine nucleotide content of isolated mitochondria. In experiments where the DPN and DPNH contents of the isolated mitochondria were measured, the mitochondria from a whole liver homogenized in 0.25 M-sucrose containing 0.03 M-nicotinamide were isolated as described above and the DPN and DPNH contents of the entire mitochondrial pellet were estimated as described by Ciotti & Kaplan (1957).

RESULTS

P/O ratios in liver mitochondria isolated from growth-hormone-treated rats. The effect of the injection of 1 mg. of growth hormone into rats 4 hr. before death on the P/O ratio of isolated mitochondria oxidizing DL- β -hydroxybutyric acid is shown in Table 1.

It is apparent that there is a considerable decrease in the P/O ratio as a result of the growth-hormone injection and that this fall can be entirely ascribed to the increase in oxygen uptake. The amount of phosphorylation is almost identical in control and treated animals. To test the effect of protein itself Armour bovine-plasma albumin was injected in amounts of up to 5 mg., but even at the highest dose used this had no effect on either oxygen uptake or the rate of phosphorylation (Table 1).

The one-step oxidation of L- β -hydroxybutyrate is equivalent to the production and oxidation of DPNH (Lehninger, 1946), and for this reason the P/O ratio of the oxidation of this latter substance by mitochondria from normal and growth-hormone-treated rats was also measured. Surprisingly, no effect was found of the growth hormone causing a lowering of the P/O ratio. The observed values were 1.77 ± 0.02 for controls and 1.79 ± 0.04 for treated rats (five rats in each group). When the P/O ratio of glutamate was measured under similar conditions, once again no effect of growth hormone on the ratio could be observed (2.31 ± 0.05 for controls and 2.29 ± 0.07 for treated rats: six rats were used in each group).

Resolution of the DL- β -hydroxybutyrate into the D- and L-salts showed that, under the conditions used here, only the L- β -hydroxybutyrate is oxidized with the D-salt being completely inactive. Thus of the three oxidation systems used here only the L- β -hydroxybutyric acid dehydrogenase is affected by growth hormone.

P/O ratios of digitonin-particles from liver mitochondria isolated from normal and growth-hormone-treated rats. A summary of the results showing the effect of pretreatment of rats with growth hormone on the rate of oxidation of DL- β -hydroxybutyrate

Table 1. P/O ratios in liver mitochondria from normal and growth-hormone-treated rats

Substrate DL- β -hydroxybutyrate. System: 0.3 ml. of 0.3M-glucose; 0.1 ml. of 0.04M-ADP; 0.1 ml. of 0.3 mM-cytochrome c; 0.5 ml. of 0.1M-sodium phosphate buffer, pH 7.4; 0.1 ml. of 0.2M-MgSO₄; 0.05 ml. of 1.5M-KCl; 0.1 ml. of 0.1M-EDTA, pH 7.4; 0.3 ml. of 0.1M-DL- β -hydroxybutyrate; 0.05 ml. of hexokinase solution ($\equiv$ 50 units of hexokinase activity); 0.2 ml. of bovine-plasma albumin (7.5 mg./ml.); 0.2 ml. of 0.02M-NaF and 1 ml. of 8% suspension of liver mitochondria. Temperature, 27°. Time, 5 min. 1.0 mg. of growth hormone or 1.0 mg. of albumin was injected 4 hr. before killing.

Treatment	No. of animals	Acetoacetate (μ mole) found (μ atom of O utilized)	Glucose 6-phosphate found (μ mole)	P/O ratio
Mitochondria				
Control	7	0.256 $\pm$ 0.02	0.663 $\pm$ 0.05	2.61 $\pm$ 0.07
Growth hormone	7	0.361 $\pm$ 0.02	0.700 $\pm$ 0.05	1.96 $\pm$ 0.06
Albumin	3	0.260 $\pm$ 0.02	0.675 $\pm$ 0.06	2.62 $\pm$ 0.07
P (hormone-treated v. controls)		0.006	Not significant	0.001
Digitonin particles				
Control	4*	0.374 $\pm$ 0.04	0.664 $\pm$ 0.05	1.78 $\pm$ 0.13
Growth hormone	4*	0.350 $\pm$ 0.04	0.616 $\pm$ 0.05	1.76 $\pm$ 0.12
P (hormone-treated v. controls)		Not significant	Not significant	Not significant

* Each sample consists of the combined livers of three rats.

Fig. 1. Effect of two batches of growth hormone on the swelling of liver mitochondria suspended in 0.25M-sucrose. Δ , 8 μ M-Inactive Somar A growth hormone; $\circ$, 2 μ M-, and $\bullet$, 4 μ M-, active growth hormone; $\blacktriangledown$, 4 μ M-thyroxine, inserted for comparison; $\blacksquare$, control curve: no additions.

by digitonin particles from rat-liver mitochondria is shown in Table 1.

It will be seen that, with DL- β -hydroxybutyrate as substrate, neither the oxygen uptake nor the rate of phosphorylation was different in the

Fig. 2. Effect of the addition of 1 mg. of various proteins to 3 ml. of a suspension of liver mitochondria in 0.25M-sucrose. $\blacktriangledown$, Bovine-plasma albumin; $\square$, casein; $\circ$, prolactin; $\bullet$, adrenocorticotrophic hormone; $\circ$, insulin; $\bullet$, growth hormone; Δ , 4 μ M-thyroxine, inserted for comparison; $\blacksquare$, control curve: no additions.

particles from the injected rats from that found in the particles of livers from control rats. It would appear that removal of the mitochondrial membrane abolished the effect of growth hormone on the efficiency of oxidative phosphorylation.

Growth hormone and mitochondrial permeability. The failure to observe an effect of growth hormone on the P/O ratio in digitonin particles drew attention to an apparent similarity between the action of growth hormone and of thyroxine in uncoupling oxidative phosphorylation, in that both hormones are active in this respect on the intact mitochondrion but had no effect on the digitonin particle. In view of the suggestion of Lehninger (1956) that the effect of thyroxine on oxidative phosphorylation was secondary to its action on mitochondrial permeability it became of considerable interest to examine whether growth hormone had an effect similar to that of thyroxine on the mitochondrial membrane.

Fig. 1 shows the effect of suspending mitochondria from a normal rat, isolated in 0.44 M-sucrose, in a medium of 0.25 M-sucrose containing growth hormone.

Growth hormone, at a concentration of $4\mu\text{M}$, causes a profound decrease in extinction. Concentrations as low as $0.02\mu\text{M}$ have also been shown to produce a quite detectable effect. On a molar basis (assuming a molecular weight of 44 000 for

growth hormone) some of the growth-hormone samples used have been about ten times as active as thyroxine.

The property of inducing swelling in isolated mitochondria appears to be closely related to the growth-promoting properties of the hormone preparation *in vivo*. When a number of batches of the hormone, of varying degrees of growth-promoting potency, were tested on isolated mitochondria, the property of causing swelling was shown only by those preparations which could cause growth of the female rat whose weight had reached a plateau value, and was only weakly present in those preparations which had a low activity in the living animal. One preparation which possessed virtually no growth-promoting activity (Armour, Somar A) actually stabilized the mitochondria either against spontaneous swelling (see Fig. 1) or against the swelling action of more active batches of growth hormone. In this respect the Armour preparation resembled the behaviour of a number of unspecific proteins such as bovine-plasma albumin, globulin, globin, lactoglobulin, casein, etc.

Of the many proteins tested, only two, insulin and adrenocorticotrophic hormone, induced swelling although both of these hormones were considerably less active than growth hormone itself (Fig. 2). If the change in extinction of the mitochondrial suspension produced by the addition of growth hormone is measured between the fifth and fifteenth minutes after mixing (corrected for spontaneous swelling) and plotted against log dose of the hormone, a typical dose-response curve is obtained (Fig. 3). Although this finding could provide the basis for a very sensitive assay of pure growth hormone it cannot, unfortunately, be used as an assay of the hormone in biological materials since the presence of small quantities of other protein (e.g. $2\mu\text{M}$ -plasma albumin) completely inhibits the promotion of swelling by growth hormone.

The resemblance between growth hormone and thyroxine both with respect to the effect on oxidative phosphorylation and on mitochondrial permeability prompted the speculation that the primary effect of growth hormone, like that of thyroxine (Lehninger, 1956), might be on the mitochondrial membrane. This point has been investigated (i) by observing whether there were changes in the permeability of mitochondria in which uncoupling had been demonstrated, and (ii) by testing whether uncoupling necessarily followed when an increased permeability was induced *in vitro*.

Mitochondrial permeability in vivo. Since mitochondria isolated from the liver of a rat treated 4 hr. previously with growth hormone exhibited a lowered P/O ratio (see Table 1) it might be expected, on the above-mentioned basis, that they

Fig. 3. log dose-response curve relating dose of growth hormone in the cuvette to change of extinction of a suspension of liver mitochondria in 0.25 M-sucrose over a period of 15 min. (Each point represents the mean of 36 determinations.)

would show increased permeability. This point was examined by comparing the degree of swelling of mitochondria, isolated in 0.44 M-sucrose, from normal and growth-hormone-treated rats when they were exposed to (a) 0.25 M-sucrose, and (b) 0.25 M-sucrose containing various concentrations of growth hormone. Under both these sets of conditions the mitochondria isolated from the hormone-treated animals behaved in a manner identical with that of controls.

It might be anticipated also that if growth hormone increased mitochondrial permeability *in vivo* the mitochondria isolated from animals so treated would be larger than those obtained from normal rats. It is not possible to compare sizes of mitochondria by direct visual methods but a comparison is possible on calculation of the extinction/mg. of mitochondrial N. (This rests on the assumption that changes of factors such as asymmetry and surface structure play only a minor role in decreasing extinction and that the principal factor involved is the change of surface area, an assumption implicit in all work on mitochondrial swelling and probably justified where, as in the present case, the mitochondria exist in spherical form.) No difference in size of mitochondria from normal and hormone-treated animals was found. The results appear to suggest therefore that mitochondria isolated from growth-hormone-treated rats are normal at least with respect to size and swelling properties.

Mitochondrial permeability and oxidative phosphorylation in vitro. It was also possible to demonstrate that mitochondria in which swelling had been induced *in vitro*, by the preincubation of mitochondria from normal rat livers with growth hormone for periods up to 4 hr. at 0° or up to 40 min. at 27°, had the same P/O ratio as did the mitochondria preincubated in the same way but in the absence of growth hormone (the values at 0° for 4 hr. were: controls 1.05; treated animals 1.10; three animals were used in each group). Addition of growth hormone *in vitro* to digitonin particles from normal rats was also without effect on the measured P/O ratio.

Pyridine nucleotide content of the isolated mitochondria. The concentration of oxidized and reduced DPN was determined in the mitochondria from normal rats and in the mitochondria from rats treated 4 hr. previously with growth hormone. The results are shown in Table 2. There is a statistically significant increase in both the total mitochondrial DPN and DPNH and in the total diphosphopyridine nucleotide expressed per gram of liver. The ratio of DPN to DPNH is also very different in the two groups of animals. In the whole liver the ratio DPN to DPNH usually found is of the order of 2 (Holzer, Goldschmidt, Lamprecht & Helmreich, 1954; Glock & McLean, 1957), but the ratio found here in mitochondria appears to be considerably higher than this. In mitochondria from growth-hormone-treated animals there is a greater proportionate increase in the DPNH than in the DPN. These findings parallel those described for the whole liver by Greenbaum & Graymore (1956). The possibility that the increased nucleotide content of the growth-hormone-treated mitochondria was due to the release of bound forms of the type described by Purvis (1958) was tested by preincubating the mitochondria of normal and hormone-treated animals with ADP for 7 min. at 30° as described by Purvis (1958). No increase in the measured pyridine nucleotide was found in either set of mitochondria.

DISCUSSION

The injection of pituitary growth hormone into normal intact rats causes a decrease in the efficiency of oxidative phosphorylation of liver mitochondria oxidizing L- β -hydroxybutyrate.

The lowered efficiency has been demonstrated in rats after the injection of 1 mg. of the hormone, an amount which raises the blood concentration by about 0.1 μ M. The normal physiological concentration of the hormone in blood has been estimated by Dr J. H. Ottaway (personal communication) to be about 0.02 μ M. Thus the amount injected is within the physiological range.

Several facts point to the site of action of growth hormone as being at the level of the mitochondrial

Table 2. Amounts of oxidized and reduced diphosphopyridine nucleotide in mitochondria from the livers of normal and growth-hormone-treated rats

Treatment	No. of animals	Wt. of liver (g.)	Total		Total DPN + DPNH (μ g.)	Total mitochondrial DPN + DPNH/g. of liver (μ g.)	DPN/DPNH
			DPN (μ g.)	DPNH (μ g.)			
Controls	6	6.54 $\pm$ 0.12	175 $\pm$ 7.1	41 $\pm$ 5.4	216 $\pm$ 8.7	32.9 $\pm$ 1.3	4.82 $\pm$ 0.73
Growth-hormone treated	6	6.27 $\pm$ 0.28	234 $\pm$ 16.4	117 $\pm$ 8.2	351 $\pm$ 21.4	55.7 $\pm$ 4.1	2.03 $\pm$ 0.17
P (controls v. hormone-treated)		0.442	0.009	< 0.001	< 0.001	0.001	0.005

membrane. First is the finding that although the P/O ratios in mitochondria isolated from normal and growth-hormone-treated animals differ, the P/O ratios in digitonin particles prepared from these mitochondria do not. Secondly, there is the activity of growth hormone as a swelling agent. Since the increased size of the mitochondria is due to the slow penetration of sucrose under the action of the swelling agent, swelling can be equated with increased mitochondrial permeability (Tedeschi & Harris, 1955, 1958). On a molar basis growth hormone seems to be at least as active as thyroxine and, further, there appears to be a close correlation between the growth-promoting activity *in vivo* and the mitochondrial-swelling activity *in vitro*. Thirdly, the idea that many hormones may have a common mechanism of action is an attractive one. In addition to growth hormone, swelling can be induced by insulin and adrenocorticotrophic hormone (although these two hormones were less effective than growth hormone). The addition of the appropriate amount of zinc (60 μ g.) did not reproduce the insulin effect. In addition to the above-mentioned hormones thyroxine (Martius & Hess, 1955) and hydrocortisone (Gallagher, 1960) share this property. Prolactin failed to induce swelling in liver mitochondria but it is possible that it would have been more effective if tested on the mitochondria of the mammary gland, its normal target organ. All the other proteins tested stabilized mitochondria against spontaneous swelling, or even against the action of hormones which would have produced swelling in their absence. All these facts point to a similarity in the mode of action of growth hormone and thyroxine.

However, some of the results obtained lead to the opposite conclusion. First, growth hormone does not uncouple oxidative phosphorylation *in vitro*, whereas this has been clearly demonstrated for thyroxine (Lehninger, Ray & Schneider, 1959; Martius & Hess, 1955). In view of the suggestion that growth hormone may be broken down to an active form *in vivo* (Park, Brown, Cornblath, Daughaday & Krahle, 1952; Bornstein, 1953) it might be held that protein growth hormone, as used here, is unsuitable for studies *in vitro*. It should be noted, however, that the intact protein hormone produces an immediate effect *in vitro* on mitochondrial permeability although it does not uncouple under identical conditions. Even increasing the level of hormone up to ten times that normally used has been found to be ineffective in changing the P/O ratio. Secondly, it was found that there was a significant fall in the P/O ratio of the mitochondria isolated from growth-hormone-treated rats, although it was not possible to demonstrate any gross changes in permeability as measured either by their subsequent response to

the addition of either growth hormone or thyroxine to the suspending medium, or by their size. Thirdly, no loss of pyridine nucleotides could be detected. Maley & Lardy (1955) have reported that there is a loss of up to 40% of the total pyridine nucleotides from the mitochondria of hyperthyroid rats, and Lehninger *et al.* (1959) found a loss of bound DPN from the interior of the mitochondria of thyroxine-treated rats. These latter authors believe that this loss could account for the lowered P/O ratios found in the mitochondria of such treated animals. This explanation does not appear to apply to growth hormone. As can be seen from Table 2 there is no loss of DPN from the mitochondria of the growth-hormone-treated rats; on the contrary, they probably contain more pyridine nucleotide than the controls. Whether this increase represents an increased synthesis or whether it is due to the liberation of a bound form of the type suggested by Purvis (1958), either by the hormone or by ADP, is not clear, but this latter alternative is made somewhat unlikely in view of the fact that preincubation with ADP made no difference to the amount of extractable DPN from either normal or growth-hormone-treated mitochondria, a result in keeping with those of Klingenberg & Slenczka (1959). The increases found in the mitochondria in the present study show a similar trend to those found in the whole liver of growth-hormone-treated rats (Greenbaum & Graymore, 1956), in that there is a greater increase in the DPNH than the DPN, with a consequent fall in the DPN/DPNH ratio.

A final difference between the action of growth hormone and thyroxine should be noted. The decrease in the P/O ratio caused by growth hormone is due to a 40% increase in the rate of oxidation with no increase in the associated phosphorylation (Table 1), whereas the uncoupling produced by thyroxine is characterized by a small increase in the rate of oxidation associated with a fall in the accompanying phosphorylation. This difference would also appear to indicate a significant difference in the way the two hormones produce their effect. It seems unlikely that the growth-hormone effect can be ascribed to a hormonally induced release of the 'R' factor of Remmert & Lehninger (1959), since this increases both respiration and phosphorylation, i.e. no change in P/O ratio, whereas other agents such as the 'U' factor (Lehninger & Remmert, 1959) and mitochrome (Polis & Shmukler, 1957) both lower the P/O ratio by decreasing the rate of phosphorylation. The increased rate of oxidation of β -hydroxybutyrate found here is of particular interest since it will be seen from Table 1 that when the mitochondrial-permeability barrier is circumvented by using digitonin particles the rate of β -hydroxybutyrate

oxidation is the same in mitochondrial fragments from normal and growth-hormone-treated rats, and, further, that both are at the same level as the enhanced value found in intact mitochondria from growth-hormone-treated animals (calculating the values for the intact mitochondria and for digitonin particles in terms of equivalent quantity of phosphorylation). This would seem to indicate the removal of an inhibition and points to the possibility that growth hormone increases the rate of penetration of the substrate. The unchanged rate of phosphorylation in all these preparations may indicate that the lowered P/O ratio found is not so much due to an uncoupling action as to the stimulation of a non-phosphorylating pathway.

The physiological significance of these findings is difficult to evaluate as a lowered efficiency of oxidative phosphorylation would be of doubtful advantage to a growing animal. It is, however, possible that, as Ernster (1956) has suggested, swelling can be regarded as a process which confers a physiological advantage on an animal. A reversible swelling of the mitochondria might be a regulatory process controlling the transfer of substrates and other materials between mitochondrion and cytoplasm. Regulation of metabolic processes by changes in permeability could well be one of the main mechanisms of hormonal activity.

SUMMARY

1. The efficiency of oxidative phosphorylation in liver mitochondria from normal and growth-hormone-treated rats has been measured with L- β -hydroxybutyrate, reduced diphosphopyridine nucleotide or glutamate as substrate. There is a decrease in the efficiency when L- β -hydroxybutyrate is used but not when reduced diphosphopyridine nucleotide or glutamate is substrate. The decreased P/O value is due to an increased rate of oxidation with no change in the rate of phosphorylation.

2. There was no difference in the P/O ratio, with L- β -hydroxybutyrate as substrate, when digitonin particles isolated from normal and growth-hormone-treated animals were compared.

3. Growth hormone at physiological concentrations produced striking increases in mitochondrial permeability when added to mitochondria suspended in sucrose.

4. The possibility that the effect of growth hormone on oxidative phosphorylation is secondary to an effect on mitochondrial permeability has been considered and the possible relationship between the effects of growth hormone and of thyroxine are discussed.

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