Biochem. J. (1966) **101**, 197

Influence of Thyroxine and Luteinizing Hormone on some Enzymes concerned with Lipogenesis in Adipose Tissue, Testis and Adrenal Gland

By J. BROWN, PATRICIA McLEAN and A. L. GREENBAUM

Department of Medicine, University of California, Los Angeles, Calif., U.S.A., Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London, W. 1, and Department of Biochemistry, University College London, Gower Street, London, W. 1

(*Received* 7 *March* 1966)

The activities of hexokinase, citrate-cleavage enzyme, 'malic enzyme' and NADP-linked isocitrate dehydrogenase have been measured in the adipose tissue, testes and adrenals of normal rats, hypophysectomized rats and hypophysectomized rats treated with either thyroxine or thyroxine plus luteinizing hormone. Hypophysectomy reduced the activity of all four enzymes in all three tissues. Thyroxine alone restored the activity of all four enzymes in adipose tissue towards normal but failed to do so in either testes or adrenals. Thyroxine and luteinizing hormone restored the citrate-cleavage enzyme activity of testes and increased the activity of hexokinase from the low value after hypophysectomy. Neither 'malic enzyme' nor isocitrate dehydrogenase was increased by thyroxine or thyroxine and luteinizing hormone in testes. The differential stimulation of enzyme activity by thyroxine in the different tissues suggests thyroxine as having a special significance in adipose-tissue lipogenesis.

Studies on regulatory mechanisms controlling metabolic pathways may best be pursued in tissues of specialized function where the general metabolic pattern has been modified to emphasize a particular pathway. This applies particularly to hormonal control where studies on specific target organs offer the advantage of a directed metabolic pattern which may be reduced to a low level by hypophysectomy, which removes the trophic hormone affecting that particular organ, and then restored to normal by injection of the appropriate hormone or stimulated by an effector hormone.

In investigating the hormonal control of lipid metabolism and the interrelationship between carbohydrate utilization and lipid formation it seemed of interest to investigate three such specialized tissues: adipose tissue, which predominantly synthesizes triglycerides, and the testis and adrenals, both of which synthesize steroids. Hypophysectomy decreases lipogenesis in all three tissues and lipogenic activity can be restored by hormonal replacement therapy.

The hormones used in the present work were thyroxine, which, in the physiological dose range, promotes lipid synthesis (Fletcher & Myant, 1958; Nejad, Chaikoff & Hill, 1962; Fain & Wilhelmi, 1962), and luteinizing hormone, which restores testicular function after hypophysectomy (Mason & Samuels, 1961; Hall & Eik-Nes, 1962; see Dorfman & Ungar, 1965). The adrenals have also been studied for comparison, as they should not be affected by either of the two hormones used. Many studies have appeared in which the response of some dehydrogenases in testis and adrenal to hormonal stimulation has been investigated, notably those by Lee & Lardy (1965), Schor, Cara & Perez (1963), Studzinski, Symington & Grant (1962), McKerns (1964) and Hilf & Burnett (1964).

In the present work four functionally related enzymes have been studied: citrate-cleavage enzyme, 'malic enzyme', NADP-linked isocitrate dehydrogenase and hexokinase. The results of Kornacker & Lowenstein (1965*a*,*b*) have indicated that changes in the activity of citrate-cleavage enzyme may be an important mechanism whereby the control of lipogenesis is effected (see also Srere, 1965), and that, in liver, the enzyme is under hormonal control. For this reason the activity of citrate-cleavage enzyme in the three tissues under different hormonal conditions has been studied. Isocitrate dehydrogenase was investigated because of the isotopic data of D'Adamo & Haft (1965), which indicate the existence of a pathway from α-oxoglutrate to citrate (and hence, via citrate-cleavage enzyme, to acetyl-CoA) in the cytoplasmic compartment of the cell. Thus this

enzyme could be an important factor in controlling lipogenesis, not only by virtue of providing reductive potential as NADPH but also as a precursor of the carbon building blocks. 'Malic enzyme' was included in this survey because of the calculations of Flatt & Ball (1964) and the findings of Pande, Khan & Venkitasubramanian (1964) and of Wise & Ball (1964) of a high activity of this enzyme in tissues with a high lipogenic activity and the correspondence of changes of the enzyme with the rate of fatty acid synthesis. Finally, the activity of hexokinase has been measured on the grounds that this is an obligatory step in carbohydrate metabolism which ultimately provides both the carbon and the hydrogen atoms for lipid synthesis.

METHODS AND MATERIALS

Animals. Hypophysectomized and control male rats were purchased from the Charles River Co. (Willmington, Mass., U.S.A.) and were maintained on stock diet supplied *ad lib.* supplemented by 5% glucose in the drinking water until used, 8 days after hypophysectomy.

Hormonal treatment. The rats were divided into four groups: (1) normal controls; (2) hypophysectomized; (3) hypophysectomized rats treated with 5μg. of L-thyroxine/day for 10 days (administered in 0·5ml. and injected subcutaneously); (4) hypophysectomized rats treated with 5μg. of L-thyroxine/day for 10 days and then for a further 4 days, on each of which they received 5μg. of L-thyroxine as above and 200μg. of luteinizing hormone as a divided dose of 100μg. morning and evening. Rats from each group were randomly selected and arranged into sets of four, each containing one rat from each of the above groups. The food intake of each set was matched, by limit-feeding, to the amount consumed by the hypophysectomized animal of the set.

Materials. L-Thyroxine was purchased from Sigma Chemical Co. (St Louis, Mo., U.S.A.). The luteinizing hormone preparation (batch no. NIH-LH-S8) was given by the Endocrinological Study Section of the National Institutes of Health, Bethesda, Md., U.S.A.) and had an activity 0·73 unit/mg. Although this preparation contained small amounts of other pituitary hormones, the only significant contamination in the present context was adrenocorticotrophic hormone (ACTH), which was present to the extent of 8·7m-units/mg. All other reagents were standard commercial products with the exception of 6-phosphogluconate dehydrogenase, which was a partially purified preparation from liver, prepared as described by Glock & McLean (1953), and used in the assay of hexokinase activity.

Nicotinamide nucleotides and CoA were obtained from C. F. Boehringer und Soehne G.m.b.H. (Mannheim, Germany).

Preparation of tissue extracts. Tissues were homogenized with an all-glass Potter–Elvehjem homogenizer in a medium containing 150mM-KCl, 5mM-$MgCl_2$, 5mM-EDTA, 10mM-mercaptoethanol and adjusted to pH7·4 by the addition of $KHCO_3$. This is similar to the extraction medium used by Sharma, Manjeshwar & Weinhouse (1963). The two epididymal fat pads from each rat were homogenized in 10ml. of medium. The testes from control rats were homogenized in 6ml. of medium and those from hypophysectomized or hypophysectomized and hormone-treated animals were homogenized in 4ml. With the adrenals, the two glands from control rats were homogenized in 4ml. of medium, but for the experimental animals the glands from two rats were pooled before homogenizing in 4ml.

The homogenates obtained as above were centrifuged at 100000*g* for 45min. and the supernatant was taken and dialysed against the extracting medium for 1hr. in the cold. This dialysed preparation was used for the determination of all the enzyme activities.

Determination of enzyme activities. (1) Hexokinase (glucose–ATP phosphotransferase, EC 2.7.1.1) was measured essentially according to the method of Sharma *et al.* (1963) with the modifications as described by McLean & Brown (1966). The final glucose concentration used was 5mM. This concentration of glucose was used because of the relatively high K_m of adipose-tissue hexokinase (DiPietro, 1963). The unit of enzyme activity is defined as the amount of enzyme catalysing the formation of 1μmole of glucose 6-phosphate/min. at 25°. (2) Citrate-cleavage enzyme [ATP–citrate oxaloacetate-lyase (CoA-acetylating and ATP dephosphorylating), EC 4.1.3.8] was estimated by the colorimetric method described by Kornacker & Lowenstein (1965*a*). (3) NADP-linked isocitrate dehydrogenase [L-isocitrate–NADP oxidoreductase (decarboxylating), EC 1.1.1.42] was measured essentially according to the method of Ochoa (1955*a*) except that the substrate concentration was doubled. (4) 'Malic enzyme' [L-malate–NADP oxidoreductase (decarboxylating), EC 1.1.1.40] was measured according to the procedure of Ochoa (1955*b*).

For these last three enzymes the unit of activity is defined as that amount of enzyme forming 1μmole of product/min. at 25°. The rate of oxidation or reduction of NADP was measured in a Unicam SP.800 recording spectrophotometer with a constant-temperature cell housing and scale-expansion accessory.

Statistical treatment. Results are expressed as units of enzyme activity $\pm$S.E.M. The differences are regarded as being significant where Fisher's P is $<0{\cdot}05$. Where P is greater than 0·1 this is shown as 'not significant' (N.S.).

RESULTS

The results showing the effects of hypophysectomy and the effects of treating hypophysectomized rats with either thyroxine or with thyroxine and luteinizing hormone on the activity of citrate-cleavage enzyme, NADP-linked isocitrate dehydrogenase, 'malic enzyme' and hexokinase in adipose tissue (the epididymal fat pad), testis and adrenal are shown in Tables 1–3. In these Tables the results are expressed on a total organ basis since the calculation of results per mg. of protein can only have limited meaning when applied to heterogeneous tissues such as adrenal and testis.

The Tables show that hypophysectomy reduces the activity of each enzyme in all three tissues. That this is due to the removal of the trophic hormones and not to the anorexia which follows

Table 1. *Effect of thyroxine and of luteinizing hormone on the activities of certain enzymes in the adipose tissue of hypophysectomized rats*

A unit of enzyme activity is defined as the amount catalysing the formation of 1 μmole of product/min. at 25°. Results are given as means ± S.E.M. Fisher's P values are given; where P is greater than 0·1 the values are quoted as N.S. (not significant). For details of treatment with thyroxine (T_4) or luteinizing hormone (LH) see the Methods and Materials section. The protein content of this tissue was (mg. of protein/epididymal fat pad): control, 4·0 ± 0·4; hypophysectomized, 2·2 ± 0·2; hypophysectomized + thyroxine, 3·0 ± 0·3; hypophysectomized + thyroxine + luteinizing hormone, 4·5 ± 0·2.

	No. of animals	Wt. of one fat pad (mg.)	Units of enzyme/epididymal fat pad: Citrate-cleavage enzyme	'Malic' enzyme	Isocitrate dehydrogenase	Hexokinase
Control	8	333 ± 13	0·31 ± 0·03	0·79 ± 0·13	0·220 ± 0·032	0·207 ± 0·036
Hypophysectomized	8	205 ± 22	0·071 ± 0·017	0·20 ± 0·03	0·130 ± 0·017	0·030 ± 0·005
Hypophysectomized + T_4	8	222 ± 18	0·34 ± 0·06	1·32 ± 0·22	0·187 ± 0·025	0·145 ± 0·010
Hypophysectomized + T_4 + LH	5	260 ± 20	0·32 ± 0·05	1·37 ± 0·28	0·315 ± 0·016	0·284 ± 0·040
Fisher's P values						
Control v hypophysectomized		< 0·001	< 0·001	0·002	0·024	< 0·001
Control v hypophysectomized + T_4		< 0·001	N.S.	N.S.	N.S.	N.S.
Control v hypophysectomized + T_4 + LH		0·013	N.S.	N.S.	0·026	N.S.
Hypophysectomized + T_4 v hypophysectomized + T_4 + LH		0·190	N.S.	N.S.	0·002	0·008

Table 2. *Effect of thyroxine and of luteinizing hormone on the activities of certain enzymes in testes of hypophysectomized rats*

A unit of enzyme activity is defined as the amount catalysing the formation of 1 μmole of product/min. at 25°. Results are given as means ± S.E.M. Fisher's P values are given; where P is greater than 0·1 the values are quoted as N.S. (not significant). Figures in parentheses are the numbers of experimental animals in each group. For details of treatment with thyroxine (T_4) or luteinizing hormone (LH) see the Methods and Materials section. The protein content of these organs was (mg. of protein/two testes): control, 501 ± 44·8; hypophysectomized, 47·1 ± 5·1; hypophysectomized + thyroxine, 43·8 ± 5·0; hypophysectomized + thyroxine + luteinizing hormone, 59·2 ± 4·7.

	Wt. of pair of testes (mg.)	Units of enzyme/two testes: Citrate-cleavage enzyme	'Malic' enzyme	Isocitrate dehydrogenase	Hexokinase
Control	2260 ± 100 (8)	0·157 ± 0·013 (8)	2·64 ± 0·35 (8)	2·29 ± 0·17 (7)	1·84 ± 0·14 (8)
Hypophysectomized	336 ± 20 (5)	0·059 ± 0·011 (5)	0·30 ± 0·04 (5)	0·32 ± 0·07 (4)	0·19 ± 0·02 (9)
Hypophysectomized + T_4	297 ± 20 (8)	0·049 ± 0·006 (8)	0·31 ± 0·06 (5)	0·35 ± 0·06 (3)	0·16 ± 0·04 (5)
Hypophysectomized + T_4 + LH	438 ± 11 (6)	0·104 ± 0·010 (5)	0·31 ± 0·05 (6)	0·43 ± 0·01 (6)	0·31 ± 0·04 (6)
Fisher's P values					
Control v hypophysectomized	< 0·001	< 0·001	< 0·001	< 0·001	< 0·001
Control v hypophysectomized + T_4	< 0·001	< 0·001	< 0·001	< 0·001	< 0·001
Control v hypophysectomized + T_4 + LH	< 0·001	0·007	< 0·001	< 0·001	< 0·001
Hypophysectomized + T_4 v hypophysectomized + T_4 + LH	< 0·001	< 0·001	N.S.	N.S.	0·015

hypophysectomy is indicated by the fact that the dietary intake was controlled in these experiments by the paired-feeding technique.

Nevertheless, although the food intake of the control and the two hormone-treated groups was limited to that of the hypophysectomized group, there may not be a strict equivalence of diet since Goodman (1964) has shown that the type of carbohydrate fed to hypophysectomized animals, as well as the amount consumed, may be critical in the control of lipid synthesis by adipose tissue.

Thyroxine treatment restored the activity of all

four enzymes in adipose tissue to normal values. This result is in accord with the finding of Tepperman & Tepperman (1964), who also reported that thyroxine was highly effective in increasing the NADP-linked malate dehydrogenase of liver. In contrast, thyroxine had no effect on any of the enzymes in testis or adrenals. Injection of 0·15 i.u. of luteinizing hormone for 4 days caused a twofold increase of citrate-cleavage enzyme and of hexokinase in testis whereas 'malic enzyme' and isocitrate dehydrogenase remained unchanged. The dose of luteinizing hormone used in these experiments was very low, less than one-third of the minimum dose used by Schor *et al.* (1963) in their study of the effect of this hormone on the testicular activity of rats and less than the 0·3 i.u./day for 7–14 days used by Arvy (1962) in her histochemical study of the testis of the fowl. In the present experiments it only increased the testis weight from 13–19% of that in hypophysectomized rats.

However, even the small dose of luteinizing hormone used here contained sufficient ACTH contaminant (1·6m-units/day) to produce significant adrenal effects. A dose of 0·05–1·0m-unit of ACTH increases the rate of secretion of compound B from the adrenal (Guillemin, Clayton, Smith & Lipscomb, 1958); and it is therefore not surprising to find that the amount of ACTH given in the luteinizing hormone preparation was sufficient to cause an increase of all three enzymes measured in the adrenals and restore them to control activities.

Luteinizing hormone increases the activity of isocitrate dehydrogenase and hexokinase in the adipose tissue of thyroxine-treated rats but has no effect on citrate-cleavage enzyme or 'malic enzyme'. Treatment with luteinizing hormone causes an increase of the protein content of epididymal fat pad (from 3·0 ± 0·3 to 4·5 ± 0·2mg./fat pad for the thyroxine and thyroxine plus luteinizing-hormone-treated rats respectively), and calculation of the enzyme activities/mg. of protein shows that luteinizing hormone does not significantly increase the activities of isocitrate dehydrogenase or hexokinase.

DISCUSSION

The reductive synthetic reactions promoted in the target organs by the administration of the appropriate trophic or effector hormones are frequently accompanied by an increase in the activity of the enzymes of the pentose phosphate pathway (Tepperman & Tepperman, 1964). Evidence in support of this view has been derived from enzyme measurements and isotope studies in a number of tissues [thyrotrophin stimulation of thyroid slices (Jarrett & Field, 1964), in adrenals after ACTH administration (Studzinski *et al.* 1962; McKerns, 1964), in the testes after gonadotrophin

Table 3. *Effect of thyroxine and of luteinizing hormone on the activities of certain enzymes in adrenal glands of hypophysectomized rats*

A unit of enzyme activity is defined as the amount catalysing the formation of 1 μmole of product/min. at 25°. Results are given as means ± S.E.M. Fisher's *P* values are given; where *P* is greater than 0·1 the values are quoted as not significant (N.S.). Figures in parentheses are the numbers of experimental observations; in the groups marked * each observation was made on the pooled material from two rats. For details of treatment with thyroxine (T_4) or luteinizing hormone (LH), see the Methods and Materials section. The protein content of these organs were (mg. of protein/two adrenals): control, 5·58; hypophysectomized, 2·0; hypophysectomized + thyroxine, 2·8; hypophysectomized + thyroxine + luteinizing hormone, 2·4.

	Wt. of pair of adrenals (mg.)	10^3 × Units of enzyme/two adrenals: Citrate-cleavage enzyme	'Malic' enzyme	Isocitrate dehydrogenase	Hexokinase
Control	31·5 ± 1·0	21·6 ± 3·3 (8)	130 ± 22 (5)	276 ± 25 (6)	58 ± 4 (7)
Hypophysectomized	10·5 ± 0·6	*5·0 ± 0·7 (5)	31 ± 6 (5)	—	*23 (2)
Hypophysectomized + T_4	11·5 ± 0·3	*6·7 ± 1·2 (4)	37 ± 7 (6)	—	*22 (2)
Hypophysectomized + T_4 + LH	13·5 ± 1·1	*33·3 ± 9·1 (3)	162 ± 12 (6)	—	*56 ± 7 (3)
Fisher's *P* values					
Control v hypophysectomized	< 0·001	< 0·001	0·004		
Control v hypophysectomized + T_4	< 0·001	0·002	0·003		
Control v hypophysectomized + T_4 + LH	< 0·001	N.S.	N.S.		
Hypophysectomized + T_4 v hypophysectomized + T_4 + LH	N.S.	0·05	0·001		

administration (Schor *et al.* 1963) and in the ovary after gonadotrophin administration (McKerns, 1965)]. Insulin causes similar changes in mammary gland (Abraham, Cady & Chaikoff, 1957; Folley & McNaught, 1958) and epididymal fat pad (Winegrad & Renold, 1958; see Jeanrenaud, 1961). In each of these examples the increased activity is an early effect and can, in many cases, be elicited by the hormones *in vitro*.

This change in the pattern of glucose oxidation is accompanied by a stimulation of fat or steroid synthesis in many tissues and it is now generally accepted that lipogenesis is associated with an increased activity of the pentose phosphate pathway. Thus a common factor in the mechanism of the stimulation of lipogenesis in target organs is the increased oxidation of glucose 6-phosphate, not only via the pentose phosphate pathway, which provides the reductive potential, but also by the glycolytic route, which provides the acetyl-CoA for the carbon 'skeleton' of fatty acids and steroids.

Hexokinase. The increased requirement for glucose 6-phosphate could be met by a number of mechanisms, such as increased phosphorylase activity, as has been shown in adrenal and ovary (Bruzzone & Brancatelli, 1965; Haynes & Berthet, 1957; see also Yates & Urquart, 1962), by an increase in the rate of glucose uptake by the target organ under the influence of hormonal treatment, as shown by Channing & Villee (1966) to occur in the ovaries of rats treated with luteinizing hormone, or by an increased rate of phosphorylation of glucose. The present experiments suggest that an increased hexokinase activity occurs when lipogenesis is stimulated. The increase of the activity of this enzyme in testis is even greater than is suggested by Table 2. The small dose of luteinizing hormone administered limits the increase of testis weight so that the total activity in the organ does not increase to any great extent. When these results are, however, calculated as activity/g. of tissue then restoration of the enzyme to normal values is virtually complete (controls, $0{\cdot}81 \pm 0{\cdot}07$; hypophysectomized + thyroxine- + luteinizing hormone-treated, $0{\cdot}71 \pm 0{\cdot}07$ μmole of glucose phosphorylated/min.). The greater specificity of thyroxine with respect to adipose tissue is illustrated by the high degree of restoration of hexokinase in this tissue when the same dose causes no restoration in either testis or adrenal. The present experiments also indicate that the known effect of thyroxine in stimulating fatty acid synthesis in the adipose tissue of hypothyroid rats (see Masoro, 1962; Brown & McLean, 1965*a*) is accompanied by an increase of hexokinase activity. Similarly, the steroidogenic effect of luteinizing hormone on testis is accompanied by a similar change, although in this case the effect is most clearly seen when the results are calculated as activity/g. of tissue (see above). The increased hexokinase observed in adrenal tissue after administration of luteinizing hormone must, presumably, be attributed to the ACTH content of the luteinizing hormone preparation and, here again, complete restoration is effected. It seems unlikely that the ACTH component of the injected luteinizing hormone produced any material effect on the testes since Schoen & Samuels (1965) have shown that 1 unit of ACTH/day administered to 3-week-old rats had no effect on androgen biosynthesis by the testis. These increases in hexokinase may be correlated with the observations of Schor *et al.* (1963) on the stimulation of glucose 6-phosphate dehydrogenase in the testes of rats treated with gonadotrophic hormones and of McKerns on the effect of gonadotrophic hormones and of ACTH on ovary (McKerns, 1965) and adrenals (McKerns, 1964) respectively where, again, the glucose 6-phosphate-dehydrogenase activity was increased. J. Brown & P. McLean (unpublished work) also noted that the low activity of the first two enzymes of the pentose phosphate pathway was increased in adipose tissue after the treatment of hypophysectomized rats with thyroxine.

Citrate-cleavage enzyme. There is considerable evidence that the activity of citrate-cleavage enzyme is an important factor in the control of lipogenesis in the liver (Kornacker & Lowenstein, 1965*a*,*b*), mammary gland (Howanitz & Levy, 1965) and adipose tissue (Kornacker & Ball, 1965; Brown & McLean, 1965*a*,*b*) and, as the present results show, a comparable situation appears to exist in the testes and adrenals. In adrenals the marked fall in activity of this enzyme is restored by thyroxine plus luteinizing hormone but not by thyroxine alone, again an effect presumably due to the ACTH contaminant. The sharp fall in the activity of citrate-cleavage enzyme in hypophysectomised rats may be correlated with the decline of steroid synthesis in the adrenal.

The data on citrate-cleavage enzyme activity in the testis, shown in Table 2, indicate, when considered on an organ basis, that the enzyme also declines in this tissue after hypophysectomy. However, the concentration of the enzyme (activity/g. of tissue) actually increases 250% since the weight of the organ decreases significantly more than the enzyme activity. Administration of luteinizing hormone increases this elevated concentration still further, but thyroxine alone has no effect. These changes take on added significance when considered in the light of the findings of Samuels & Helmreich (1956) on the steroid 3-β-ol dehydrogenase of testis. The concentration of this last-named enzyme in hypophysectomized rats, after an initial decline, increases slowly and reaches

concentrations well above normal by the twentieth day after hypophysectomy, i.e. the same stage as the animals used in this study. The close parallelism between the activity of citrate-cleavage enzyme and steroid 3-β-ol dehydrogenase through the same somewhat unusual course and the preferential preservation of citrate-cleavage enzyme indicates the involvement of citrate-cleavage enzyme in steroid synthesis.

Lipogenesis is sharply reduced in the adipose tissue of hypophysectomized animals (see Masoro, 1962) and this is, in a large part, due to a lack of thyrotrophin (Fain & Wilhelmi, 1962). In this tissue, again, the changes in citrate-cleavage enzyme parallel the known changes in the rate of lipogenesis. The results given here extend the views of Srere (1965) and of Lowenstein (1964) by showing that citrate-cleavage enzyme is not only important in tissues synthesizing fatty acids but may also play a similar role in tissues making steroids.

'*Malic enzyme*'. The role of 'malic enzyme' in lipogenesis has recently received renewed attention in view of the calculations of Flatt & Ball (1964) and of Landau, Katz, Bartsch, White & Williams (1965) that the NADPH generated by the pentose phosphate pathway is only about 50% of that needed for fatty acid synthesis and that the remainder could be derived from NADP-linked 'malic enzyme', and of the suggestion by Lowenstein (1964) and by Wise & Ball (1964) that this enzyme might also be involved in the return of the oxaloacetate, produced by citrate-cleavage enzyme in the cytoplasmic compartment, to the mitochondrion. In adipose tissue and adrenals the changes in 'malic enzyme' parallel those of citrate-cleavage enzyme. No such parallelism occurs in the testis. In this tissue citrate-cleavage enzyme appears to be preferentially maintained when the testis decreases in weight but the 'malic enzyme' decreases in proportion to the tissue weight. It may be noted that, as shown by Rudolph & Olsen (1956), the two dehydrogenases of the pentose phosphate pathway also decrease in proportion to the tissue weight and are comparable in activity to the 'malic enzyme'. It is, nevertheless, clear that even at these low levels they are in considerable excess over the rate at which acetyl-CoA can be produced by citrate-cleavage enzyme. The relative excess of 'malic enzyme' over citrate-cleavage enzyme varies widely from tissue to tissue. In adipose tissue this ratio is 2·5; in adrenal it is 6 and in testis it is 17.

The data presented here emphasize the significant role played by thyroxine in adipose-tissue metabolism. Under conditions where thyroxine restores the activity of some enzymes concerned with lipid synthesis in the adipose tissue of hypophysectomized animals it has no effect on the same enzymes in adrenals or testes, whereas the appropriate hormones cause at least some measure of restoration of the enzymes in these two tissues.

We thank the British Empire Cancer Campaign for financial support, the U.S. Public Health Service for the award of a Fellowship to J. B. while working at the Middlesex Hospital Medical School, the Medical Research Council for the purchase of the Unicam spectrophotometers (P. M. and A. L. G.) and Mr K. Greenslade for skilled technical assistance.

REFERENCES

Abraham, S., Cady, P. & Chaikoff, I. L. (1957). *J. biol. Chem.* **224**, 955.
Arvy, L. (1962). *C.R. Soc. Biol.* **156**, 1802.
Brown, J. & McLean, P. (1965*a*). *Biochem. biophys. Res. Commun.* **21**, 607.
Brown, J. & McLean, P. (1965*b*). *Nature, Lond.*, **207**, 407.
Bruzzone, S. & Brancatelli, A. (1965). *Endocrinology*, **77**, 423.
Channing, C. P. & Villee, C. A. (1966). *Biochim. biophys. Acta*, **115**, 205.
D'Adamo, A. F. & Haft, D. E. (1965). *J. biol. Chem.* **240**, 613.
DiPietro, D. L. (1963). *Biochim. biophys. Acta*, **67**, 305.
Dorfman, R. I. & Ungar, F. (1965). *Metabolism of Steroid Hormones*, p. 138. New York: Academic Press Inc.
Fain, J. N. & Wilhelmi, A. E. (1962). *Endocrinology*, **71**, 541.
Flatt, J. B. P. & Ball, E. G. (1964). *J. biol. Chem.* **239**, 675.
Fletcher, K. & Myant, N. B. (1958). *J. Physiol.* **144**, 361.
Folley, S. J. & McNaught, M. L. (1958). *Brit. med. Bull.* **14**, 207.
Glock, G. E. & McLean, P. (1953). *Biochem. J.* **55**, 400.
Goodman, H. M. (1964). *Endocrinology*, **75**, 140.
Guillemin, R., Clayton, E. W., Smith, J. D. & Lipscomb, H. S. (1958). *Endocrinology*, **63**, 349.
Hall, P. F. & Eik-Nes, K. (1962). *Biochim. biophys. Acta*, **63**, 411.
Haynes, R. C. & Berthet, L. (1957). *J. biol. Chem.* **225**, 115.
Hilf, R. & Burnett, F. F. (1964). *Arch. Biochem. Biophys.* **104**, 106.
Howanitz, P. J. & Levy, H. R. (1965). *Biochim. biophys. Acta*, **106**, 430.
Jarrett, R. J. & Field, J. B. (1964). *Endocrinology*, **75**, 711.
Jeanrenaud, B. (1961). *Metabolism*, **10**, 535.
Kornacker, M. S. & Ball, E. G. (1965). *Proc. nat. Acad. Sci., Wash.*, **54**, 899.
Kornacker, M. S. & Lowenstein, J. M. (1965*a*). *Biochem. J.* **94**, 209.
Kornacker, M. S. & Lowenstein, J. M. (1965*b*). *Biochem. J.* **94**, 832.
Landau, B. R., Katz, J., Bartsch, G. E., White, L. W. & Williams, H. R. (1965). *Ann. N.Y. Acad. Sci.* **131**, 43.
Lee, Y. P. & Lardy, H. A. (1965). *J. biol. Chem.* **240**, 1427.
Lowenstein, J. M. (1964). *I.U.B. Symposium Series: Oxygen in the Animal Organism*, vol. 31, p. 163.
McKerns, K. W. (1964). *Biochim. biophys. Acta*, **90**, 357.
McKerns, K. W. (1965). *Biochim. biophys. Acta*, **97**, 542.
McLean, P. & Brown, J. (1966). *Biochem. J.* **98**, 874.

Mason, N. R. & Samuels, L. T. (1961). *Endocrinology*, **68**, 899.
Masoro, E. J. (1962). *J. Lipid Res.* **3**, 149.
Nejad, N. S., Chaikoff, I. L. & Hill, R. (1962). *Endocrinology*, **71**, 107.
Ochoa, S. (1955*a*). In *Methods in Enzymology*, vol. 1, p. 699. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
Ochoa, S. (1955*b*). In *Methods in Enzymology*, vol. 1, p. 739. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
Pande, S. V., Khan, P. R. & Venkitasubramanian, T. A. (1964). *Biochim. biophys. Acta*, **84**, 239.
Rudolph, C. G. & Olsen, N. S. (1956). *Biochim. biophys. Acta*, **19**, 382.
Samuels, L. T. & Helmreich, M. L. (1956). *Endocrinology*, **58**, 435.
Schoen, E. J. & Samuels, L. T. (1965). *Acta endocr., Copenhagen*, **50**, 365.
Schor, N. A., Cara, J. & Perez, A. (1963). *Nature, Lond.*, **198**, 1310.
Sharma, R. M., Manjeshwar, R. & Weinhouse, S. (1963). *J. biol. Chem.* **238**, 3840.
Srere, P. A. (1965). *Nature, Lond.*, **205**, 766.
Studzinski, G. P., Symington, T. & Grant, J. K. (1962). *Acta endocr., Copenhagen*, **40**, 232.
Tepperman, H. M. & Tepperman, J. (1964). *Amer. J. Physiol.* **206**, 357.
Winegrad, A. I. & Renold, A. E. (1958). *J. biol. Chem.* **233**, 273.
Wise, E. M., jun. & Ball, E. G. (1964). *Proc. nat. Acad. Sci., Wash.*, **52**, 1255.
Yates, F. E. & Urquart, J. (1962). *Physiol Rev.* **42**, 388.