essentially the same picture; an initial depression of the respiratory quotient which was maintained in the case of the free-fed animals but which, in the case of the limit-fed group, rose to the control levels after 50 days of treatment.

5. The results are believed to demonstrate that protein deposition in growth-hormone-treated rats can only take place as a result of an increased emphasis on fat catabolism, with a consequent sparing of dietary proteins for deposition in the tissues. As a corollary to this, it has been shown that in limit-fed rats growth ceases when the body stores of labile fat are exhausted. It is suggested that one of the primary effects of growth hormone activity is in the catabolism of fat.

I should like to thank Prof. F. G. Young for his encouragement and advice, and Miss P. McLean for her helpful criticism. I should also like to acknowledge my great debt to Miss E. I. Large for her invaluable assistance.

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The Mobilization of Lipid by Anterior Pituitary Growth Hormone

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Injections of growth-promoting anterior pituitary extracts into rats have been shown to increase the nitrogen and decrease the fat content of the carcass (Lee & Schaffer, 1934; Young, 1945). Similar effects have been demonstrated with purified growth hormone (Li, Simpson & Evans, 1948; Greenbaum, 1953). The importance of pituitary growth hormone in controlling fat metabolism has also been indicated by the lowering of the respiratory quotient (Gaebler, 1933*a*, *b*; Greaves, Freiberg & Jones, 1940; Greenbaum, 1953), and by the massive and rapid mobilization of fat to the liver found after injections of the hormone. Doses as low as 100 μg. of purified growth hormone have been shown by Weil & Ross (1949) to cause a significant increase in the liver fat of mice within 2 hr. after treatment and a doubling of the control level after 7 hr. Li, Simpson & Evans (1949) observed a similar increase when they examined the effect of acute treatment with growth hormone (5 mg. administered over a period of 6 hr.) in rats previously starved for 24 hr. In these animals the liver fat was increased by 65 % in 6 hr. These authors also reported that when the treatment was extended over periods longer than 10 days this effect was reversed, and the fat content of the liver was always decreased. They suggested that growth hormone first stimulated a mobilization of fat to the liver and subsequently increased its rate of oxidation.

Barrett, Best & Ridout (1938) and Stetten & Salcedo (1944) have used deuterium to label the depot fats of the body, and have shown from the rate at which deuterated fats accumulated in the liver after injections of growth-promoting pituitary extracts, that the primary source of this new lipid was the body depots. There have been relatively

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few experiments concerned with the pattern of the change and distribution of the lipids, over a period of treatment with growth hormone. One such experiment was that of Campbell & Lucas (1951) who administered anterior pituitary extracts to mice previously fasted for 4 hr. and measured the distribution of lipids 7 and 20 hr. later. They found an increase in the liver lipids at both of these time intervals and showed that this was largely accounted for by an increase in the neutral fat fraction. At the same time the triglyceride content of the adipose tissue, carcass and skin was found to be reduced.

The source of this mobilized fat has been studied only in experiments which employed crude pituitary extracts. The results of such experiments are not unequivocal since such extracts frequently contain adrenocorticotrophic hormone (ACTH) in addition to growth hormone (Campbell & Davidson, 1951). Since ACTH also has striking effects on the mobilization of fat to the liver (Li *et al.* 1949), it follows that the effects observed after administration of crude pituitary extracts may not necessarily be entirely attributable to their growth hormone content. The interpretation is further complicated by the use by many investigators of starved animals.

The present investigation was designed to measure the changes in neutral fat, phospholipin, free and combined cholesterol, using a highly purified hormone preparation, not only over short time intervals of a few hours, but also over much longer periods, up to 8 days, during which it has been shown that growth is proceeding and fat is being catabolized at a high rate (Greenbaum, 1953). A knowledge of the pattern of change in the distribution of lipids, both in the blood and liver, was felt to be a necessary preliminary in the study of the influence of growth hormone on fat metabolism.

EXPERIMENTAL

Animals. Female hooded Norway rats of the Medical Research Council strain, aged approximately 6 months and weighing between 170 and 200 g. were used. The rats were kept in individual cages and fed a constant daily amount (22 g.) of the diet described previously (Greenbaum, 1953). This regimen was begun 7 days before the commencement of the experiment to allow full equilibration to the new dietary conditions, and was maintained throughout the experimental period. This was the average daily amount of food which was voluntarily eaten by these adult normal female rats in the absence of growth hormone treatment.

Biological methods. The rats were randomly distributed among eight groups, each group consisting of six animals; one group of controls and seven groups treated with growth hormone for different periods of time, ranging from 3 hr. to 8 days. Rats treated for 3, 6 or 12 hr. received a single intraperitoneal injection of 0·5 mg. of growth hormone at the stated time interval prior to killing. Animals treated for longer periods received daily subcutaneous injections of 0·5 mg. of growth hormone at 9 a.m., the injection on the final day being intraperitoneally. The growth hormone used was a highly purified material, fraction A, prepared by the method of Wilhelmi, Fishman & Russell (1948) which, for injection purposes, was suspended in water and the pH adjusted to 8·0 to ensure solution. Growth hormone prepared by the method of Wilhelmi *et al.* (1948) is claimed by the authors to contain not more than 5 parts in 10000 of ACTH, so that, at the dose level used in this experiment, less than 0·2 μg. ACTH was administered as a contaminant of the growth hormone. Control animals were injected with 0·5 ml. of saline.

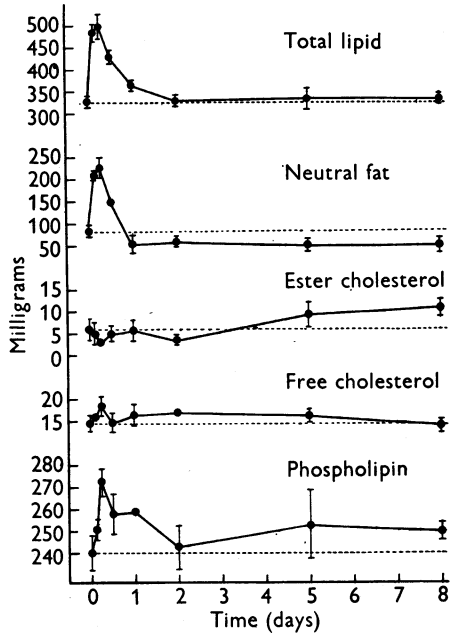

Fig. 1. The effect of treatment with growth hormone for different lengths of time on the distribution of lipids in the entire rat liver. The horizontal dotted lines represent the control levels. The vertical lines are equal to twice the S.E.M. Where no vertical line is given the S.E.M. is too small to show.

Blood samples were obtained under sodium amytal anaesthesia. Between 5 and 7 ml. of blood were withdrawn into a heparinized syringe from the dorsal aorta. The liver sample was always taken from the median lobe.

Chemical methods. The techniques used for the estimation of the lipids were according to the methods of Bloor as modified by Boyd (1938). The oxidative method there described was used for the estimation of the total fatty acids and total and free cholesterol. It was found more convenient, however, to measure the phospholipin by digesting this fraction in perchloric acid and then measuring the inorganic phosphorus colorimetrically by the method of Allen (1940), using a factor of 25·1 to convert the phosphorus values to phospholipin. From the values obtained from these four fractions it was possible to calculate the distribution of the lipids.

RESULTS

The experimental results are illustrated in Figs. 1 and 2 and in the histogram, Fig. 3.

The most striking change is the increase in total lipid both of the liver and plasma 3–6 hr. after administration of growth hormone. This is followed by an equally rapid decline in the plasma level, but in the liver the decline is slower, only reaching control levels 24 hr. after treatment of the animal with the hormone. It remained at the control level throughout the remainder of the experiment in spite of continued injections of growth hormone.

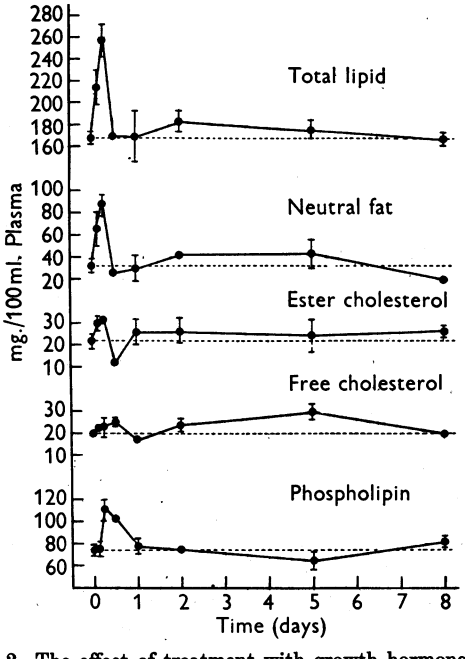

Fig. 2. The effect of treatment with growth hormone for different lengths of time on the distribution of lipids in rat blood plasma. The horizontal dotted lines represent the control levels. The vertical lines are equal to twice the S.E.M. Where no vertical line is given the S.E.M. is too small to show.

After these initial changes the values for the total lipid of the plasma and the total lipid of the entire liver do not differ significantly from the control values.

The changes with time in the distribution of the lipids in the plasma and liver are best seen in the form of a histogram, Fig. 3, which illustrates the percentage increase of the total lipid of the experimental animals over that of the controls, and the partition of this increment among the various lipid fractions.

Plasma. Three hours after growth-hormone treatment the plasma neutral fat had markedly increased. The increase of 33 mg. of neutral fat/100 ml. of plasma accounts for 71·5 % of the observed rise in the total lipid, the remainder being almost entirely due to the rise in the ester cholesterol fraction. It is notable that no change in the phospholipin had occurred. Six hours after injection of growth hormone the percentage increase in total lipid was double that found at 3 hr. The ester cholesterol was still elevated above the control level and there had been a dramatic rise in the phospholipin fraction. After this time the total lipid value declined towards the control value. Under the conditions of this

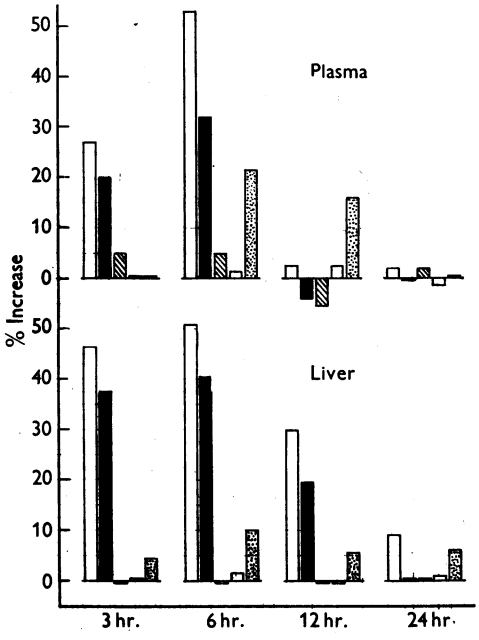

Fig. 3. The pattern of lipid distribution in rat blood plasma and entire liver. The first white column represents the percentage increase of total lipid over the control value. The remaining four columns are, in order, neutral fat, ester cholesterol, free cholesterol and phospholipin and show the relative contribution that each makes to the increased total lipid.

experiment the peak in the mobilization of the lipids occurred 6 hr. after administration of the growth hormone.

Although the plasma total lipid had returned almost to the control value by 12 hr. the pattern of the distribution of the lipids found at this time interval differed in many respects from that observed in the controls. The neutral fat had returned to control level or was even slightly below it. The ester cholesterol was significantly lower than normal and concomitant with this there was a rise in the free cholesterol value. On the other hand, the phospholipin was still very much above control

value. Twenty-four hours after the injection the level of all plasma lipid fractions had returned to normal and for the remainder of the experimental period remained close to this level.

Liver. The changes described below are the changes in the various lipid components calculated on the basis of the entire liver. Three hours after the injection of growth hormone the total neutral fat of the liver had risen from 83·6 mg. to 208·1 mg. and the neutral fat therefore accounts for 80·5 % of the total lipid mobilized, the remainder being accounted for by the increased phospholipin. The pattern of the distribution is not materially changed during the next 3 hr., the only outstanding difference being the increased phospholipin. At this time, and indeed throughout the experiment, the total liver free and ester cholesterol showed no very marked changes.

The liver total lipid had reached a maximum by the sixth hour and thereafter declined towards the control values, although this decrease was slower than that found in the plasma. At 12 hr. the total lipid was still elevated above normal and this was due to a maintenance of a higher level of the neutral fat and phospholipin fractions. The phospholipin was the only lipid constituent that had still not returned to control values at 24 hr. A complete restoration of the normal picture was not attained until 48 hr. after growth-hormone treatment. Thereafter all fractions remained substantially at control level, although there was a tendency for the total neutral fat to fall below, and the total liver ester cholesterol to increase above, this level.

The changes in liver constituents so far considered have referred to the total quantity of the lipid present in the liver. However, when these results are expressed on a basis of mg. lipid/g. of liver two differences emerge. First, in contrast with the unchanged level of the total lipid found in the entire liver after 1, 2 and 5 days of treatment with growth hormone, the total lipid/g. of liver falls below the control value at these time intervals. Secondly, there is no increase in phospholipin (mg./g. of liver) to parallel the increase found in the entire liver. Both of these observations may be accounted for by the increase in liver weight which was observed in growth-hormone-treated rats.

DISCUSSION

The results presented here on the rapid mobilization of lipids to the liver in response to growth-hormone treatment are in accord with those of Li *et al.* (1949), Szego & White (1949), Weil & Ross (1949) and Campbell & Lucas (1951). However, in contrast with the results reported by Campbell & Lucas (1951), who found that the elevated level of total lipid and neutral fat in the livers of starved mice treated with anterior pituitary extract was maintained for at least 20 hr. after treatment, in the present investigation the peak of mobilization had been passed at 12 hr. and the level of these lipids was within the control range by 24 hr. It is probable that this difference can be accounted for by the diverse conditions used in the two experiments.

While it is realized that the changes in concentration of lipids alone does not necessarily show the role they play in the mobilization of fat, yet the large changes occurring shortly after growth-hormone treatment may well reflect the initial steps involved in this process. The increased total lipid found in the plasma and liver in the first few hours after treatment with growth hormone can be largely accounted for, on a quantitative basis, by the striking increase in the neutral fat fraction. By the time the first measurements were taken, 3 hr. after growth-hormone treatment, the plasma neutral fat had doubled and there had been a rise, albeit smaller, in the ester cholesterol fraction, but no increase in the phospholipin was detected. In the liver, also, there was a dramatic rise in the neutral fat, although the changes in ester cholesterol and phospholipin were insignificant. By 6 hr. the plasma neutral fat had reached its peak and the liver neutral fat was still maintained at a high level.

The coincident increase in the neutral fat content of the plasma and liver after such a short time interval would appear to offer some evidence that, under the conditions of these experiments, the primary transport of fat from the depots to the liver may occur in the form of neutral fat. The rise in plasma ester-cholesterol may also indicate the implication of cholesterol esters in this process, and similarly the failure to detect any change in the phospholipin at this stage tends to throw some doubt on the participation of this fraction in the initial mobilization. This conclusion derives support from the observations of Hodge *et al.* (1941), that when fat is mobilized to the liver under conditions such as fasting, it is taken without selection from the fat depots, and that while the liver neutral fat and cholesterol are markedly increased the phospholipin fraction remains constant. Furthermore, Frazer (1949), in discussing the transport of lipid in the animal body, concludes that lipids are transported mainly as long-chain triglycerides in particulate form, both in the movement of fat from the intestinal cells, via the lymph, to the systematic blood and fat depots, and in the mobilization of fat from the depots to the liver. While the observations reported here do not provide direct evidence for the form in which fat is mobilized under the influence of growth hormone, the similarity of pattern between the initial stages of mobilization in growth-hormone-treated rats and that observed during fasting, suggests that in the present investigation some of the fat mobilized to

the liver may well have been in the form of particulate triglyceride.

The conclusion that plasma cholesterol also plays a part in this mobilization is in keeping with the observation of Thannhauser & Schmidt (1946) that whenever neutral fat increases in serum, cholesterol rises *pari passu*.

A point of considerable interest is the fact that although the neutral fat of the plasma continued to rise between the third and sixth hour after administration of growth hormone, the liver neutral fat had reached its maximum at 3 hr. and no further increment occurred in the following 3 hr. This observation is of some importance in view of the fact that during the second 3 hr. interval the plasma phospholipin had risen dramatically and a marked increase in the liver phospholipin was also observed. These findings suggest that at this time while mobilization of fat from the depots is still occurring, as evidenced by the high plasma neutral fat and ester cholesterol, the liver is synthesizing phospholipin from neutral fat at an increased rate, and a proportion of this phospholipin is being liberated into the plasma. If this process were in fact occurring, then it is perhaps not surprising that the liver neutral fat content does not increase despite the elevated plasma neutral fat.

Two further lines of evidence may be adduced in support of the suggestion that the liver neutral fat acts as a source of the plasma phospholipin. First, as Fig. 3 clearly illustrates, there is an increase in the liver phospholipin at 3 hr. and a further increment at 6 hr., while no increase in plasma phospholipin is found until the sixth hour. Secondly, evidence is presented in the following paper (Greenbaum & McLean, 1953) that 6 hr. after growth-hormone treatment liver fatty-acid oxidase is greatly reduced. Oxidation of the neutral fat cannot, therefore, be held to account for the lack of parallelism between the plasma and liver neutral fat.

Ample evidence is available in the literature showing that the liver is indeed the source of the plasma phospholipin. Fishler, Entenman, Montgomery & Chaikoff (1943) and Goldman, Chaikoff, Reinhardt, Entenman & Dauben (1950), using hepatectomized dogs, have shown that the rate of plasma phospholipin formation was negligible in the absence of the liver, compared with the rate in normal controls. Further, Pihl & Bloch (1950), using labelled acetic acid in normal rats, observed that the concentration of isotope in the fatty acids of plasma phospholipin was exceeded only by that of the liver fatty acids, and concluded that the plasma phospholipin must have originated from this organ. Similar lines of evidence have pointed to the liver as a source of plasma cholesterol (Popják & Beeckmans, 1950; Gould *et al.* 1951). Geschwind, Li & Evans (1950) have presented evidence to show that the formation of phospholipin is under hormonal control. They found that hypophysectomy resulted in a decrease in the turnover rate of liver phospholipin, while treatment with growth hormone increased the turnover rate, both in normal and hypophysectomized rats. While these authors made no measurements at time intervals less than 14 days, their observations nevertheless lend support to the hypothesis that shortly after growth-hormone treatment phospholipin synthesis in the liver is greatly stimulated.

It is pertinent to consider here the possibility that the phospholipins serve in the transport of fatty acids from the liver to the extrahepatic tissues. The rapid fall in plasma phospholipin between 12 and 24 hr. after growth-hormone treatment, at a time when the liver phospholipin is still much higher than the control value, may indicate the transport to, and the utilization by, the extrahepatic tissues. There is, however, no general consensus of opinion whether, in fact, the phospholipins do play such a role. The experiments of Zilversmit, Entenman, Fishler & Chaikoff (1943) showed that the extrahepatic tissues took up only 10 % of an administered dose of labelled phospholipins and Entenman, Chaikoff & Zilversmit (1946) demonstrated that the rate of removal of labelled phospholipin from the plasma was greatly reduced in the absence of the liver. However, the conditions employed by these investigators are so greatly at variance with those used here that their conclusion that phospholipins play only a minor role in the transport of fat from the liver, may not necessarily apply to the present experiment.

A second phase in the picture of the distribution of the lipids is the general decline to control values during the second 12 hr. It is possible that the decline in neutral fat may be associated with the increase in fatty-acid oxidase activity which occurs at this time, and in fact it can be calculated that the increased activity of this enzyme could largely account for the decreased neutral fat content of the liver (Greenbaum & McLean, 1953). It is probable that this is not the only factor. Decreased mobilization, as evidenced by the fall in plasma neutral fat and ester cholesterol and the rise in free cholesterol, or an increased extrahepatic utilization, could also contribute to this decline. The more rapid decline of neutral fat compared with phospholipin during this phase may be explained by the faster rate of regeneration of the neutral fat of the liver as shown by Pihl & Bloch (1950).

There is a third and final phase during which all values for the various lipid components, both for plasma and liver, remain substantially within the range of the control values. However, this must not be interpreted as showing that the rate of the metabolism of the lipids has also returned to normal.

In fact this cannot be the case since, as shown in a previous communication (Greenbaum, 1953), the oxidation of fat, as indicated by the respiratory quotient, is greatly stimulated at this time. This illustrates the difficulty of translating absolute values into terms of metabolic rates, a difficulty which is exemplified by the experiments of Hodge *et al.* (1941), who demonstrated that while approximately equal amounts of fat were mobilized to the liver on the first and second days of fasting, an accumulation of neutral fat occurred only on the first day, as by the second day the enzyme systems were so adjusted that no excess fat remained. A further illustration is to be found in the experiments of Geschwind *et al.* (1950) in which it was demonstrated that whereas neither hypophysectomy nor hypophysectomy plus growth hormone changes the concentration of liver phospholipin in rats, yet both of these conditions had significant results on phospholipin turnover rates. It is clear, therefore, that the lack of any very marked changes in the lipid concentration of rats treated for long periods with growth hormone must be interpreted with caution.

Comparisons must be incomplete because of the many processes occurring in the liver, fat depots, blood and extrahepatic tissues with respect to fat mobilization, transport and oxidation, and an adequate picture could only be achieved by considering not only absolute values but also turnover rates. For a deeper understanding of the influence of growth hormone on these processes it is necessary to study the effect of the hormone on the enzymes involved. As a first step experiments have been initiated on the measurement of the level of certain enzyme systems involved in fatty-acid catabolism using identical conditions of growth-hormone treatment as those described here. Further consideration of the present data has therefore been deferred to the discussion of these results (Greenbaum & McLean, 1953).

SUMMARY

1. The distribution of the lipids of liver and plasma of normal, adult, female rats, treated for differing periods of time with purified growth hormone, has been investigated.

2. Growth hormone causes a rapid mobilization of lipid to the liver, the peak of mobilization occurring 6 hr. after treatment with the hormone.

3. Transport to, and accumulation in, the liver is mainly a result of changes in the neutral fat fraction of the lipids. It is possible that cholesterol esters also play a part in this mobilization.

4. Evidence is presented that 6 hr. after growth-hormone treatment, the synthesis of phospholipins by the liver is stimulated and that these are then released to the plasma.

5. The values for the various lipid fractions have all returned to the control level by 24 hr. after growth-hormone treatment.

6. The significance of these results in the metabolism of fat under the influence of growth hormone is discussed.

Since this paper was written a comprehensive review of the hormonal factors affecting the mobilization of fat has been published by Levin & Farber (1952). Their conclusion, that a pituitary factor, probably growth hormone, acts as a triggering substance in the mobilization of fat, is in keeping with the conclusions outlined in the present communication.

We wish to express our gratitude to Prof. F. G. Young for his interest in this work and to Miss E. I. Large for her valuable assistance. It is a pleasure to acknowledge our debt to the Medical Research Council for a training grant to one of us (P.M.) during the tenure of which this work was done.

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The Influence of Pituitary Growth Hormone on the Catabolism of Fat

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The increase in nitrogen and water and the decrease in fat content of animals treated with growth-promoting anterior pituitary extracts (Lee & Schaffer, 1934; Young, 1945; Li, Simpson & Evans, 1948), and the lowering of the respiratory quotient (Gaebler, 1933*a*, *b*; Greaves, Freiberg & Jones, 1940; Greenbaum, 1953), clearly indicate that the metabolic pattern of such treated animals is changed, so that a larger proportion of the energy requirement is obtained from the oxidation of fat. Two further lines of evidence point to this same conclusion. The mobilization of fat to the liver within 6 hr. and its subsequent disappearance during the following 18 hr. (Szego & White, 1949; Li, Simpson & Evans, 1949; Greenbaum & McLean, 1953) and the increased level of blood and urinary ketones in rats shortly after growth-hormone treatment (Bennett, Kreiss, Li & Evans, 1948; Bondy & Wilhelmi, 1950) are both indicative of an increased fat catabolism.

There is little information available to indicate whether the influence of growth hormone is on the catabolism of fat in the liver. Bondy & Wilhelmi (1950) have shown that the acute treatment of fasted rats with massive doses of growth hormone failed to increase the rate of acetoacetate production of liver slices from such treated animals, although they did observe an increase in the level of blood ketones. The same authors also found that ketone production by liver slices from hypophysectomized rats, incubated *in vitro* in the absence of added substrate, was greatly reduced, and Tepperman & de Witt (1951), using similar methods, have confirmed this result. Shipley (1944) has claimed that the addition of relatively crude pituitary extracts to liver slices incubated in serum resulted in an increased acetoacetate production, but Bondy & Wilhelmi (1950) have been unable to confirm this observation using purified growth hormone. There has been no report of the effect of growth hormone on the degradation of fatty acids in the liver.

The influence of growth hormone on the extrahepatic utilization of fat and ketone bodies has also received scant attention. While muscle tissue is known to oxidize ketone bodies rapidly (Harrison & Long, 1940; Stadie, 1945) the evidence of Bennett *et al.* (1948) showing that growth hormone failed to influence their rate of utilization in eviscerated, nephrectomized rats, seems to indicate that this hormone does not stimulate extrahepatic utilization. The direct oxidation of long-chain fatty acids by tissues other than the liver has only recently been conclusively demonstrated (Geyer, Waddell, Pendergast & Yee, 1951; Weinhouse, Millington & Volk, 1950) but, so far, the possible hormonal control of this aspect of fat metabolism has attracted little attention.

A quantitative assay of the overall activity of the liver fatty acid-oxidase system would throw considerable light on the problem of the locus of action of growth hormone in increasing fat oxidation in treated animals and such a study has therefore been initiated. Furthermore, it was of interest to relate the changes in the activity of this enzyme system with the observed mobilization and subsequent loss of neutral fat in the liver. Measurements were also made of the acetoacetate production by the liver since the ketones produced may serve as fuel for the extrahepatic tissues, and because the correlation of liver acetoacetate production with the measured *in vivo* changes in blood and urine ketone bodies is of some interest.

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