produced a rise in blood pressure, probably through an ephedrine-like action. Its potency was about a fifth of that of ephedrine and it also caused tachyphylaxis. On the other hand, the sympathomimetic activities of 3-(2-amino-1-hydroxypropyl)pyridine (IV) resembled more those of adrenaline and noradrenaline. In addition, this compound stimulated autonomic ganglia.

DISCUSSION

It has been shown that norephedrine is as effective as ephedrine in reducing adrenaline hyperglycaemia. It is possible therefore that the failure of 3-(2-amino-1-oxopropyl)pyridine and 3-(2-amino-1-hydroxypropyl)pyridine to act similarly is not due to the absence of an *N*-methyl group in the side chain. Nor can the lack of activity of these compounds be attributed to the *iso*propylamine side chain that is present in all four amines. Since the hydroxypropyl derivative differs from norephedrine only in the substitution of a pyridine for a benzene ring, it is evident that some alterations to the ring moiety of ephedrine analogues interfere with their adrenaline-hyperglycaemia-blocking activity.

Although norephedrine has no hyperglycaemic activity, that of its catechol analogue, cobefrin, is very marked (Schaumann, 1931; Anderson & Chen, 1934). In the present work the pyridine analogue of cobefrin and norephedrine, 3-(2-amino-1-hydroxypropyl)pyridine, has been shown to exhibit no hyperglycaemic activity. While the substitution of a pyridyl for a dihydroxyphenyl radical abolished the hyperglycaemic effect, the blood-pressure reaction of the ephedrine type of amine was replaced by one more characteristic of adrenaline and noradrenaline. The effect of substituting the side chain at the 4-position on the pyridine ring could not be determined owing to the difficulty of obtaining the required amines.

SUMMARY

1. Ephedrine and norephedrine significantly reduced adrenaline-induced hyperglycaemia in rats.

2. 3-(2-Amino-1-oxopropyl)pyridine and 3-(2-amino-1-hydroxypropyl)pyridine did not change the blood-sugar level of rats or affect the hyperglycaemia produced by adrenaline. Both compounds exhibited sympathomimetic activity.

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The Synthesis of Lipids in the Livers of Rats Treated with Pituitary Growth Hormone

By A. L. GREENBAUM
Department of Biochemistry, University College London

and R. F. GLASCOCK
National Institute for Research in Dairying, University of Reading

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Studies on the carcass composition of rats treated with pituitary growth hormone (Lee & Schaffer, 1934; Young, 1945; Greenbaum, 1953) have led to the conclusion that the hormone exerts a profound influence on the course of fat metabolism.

Growth hormone injected into the intact rat stimulates fat catabolism in the liver, but this alone is not considered sufficient to account for the decline in total body fat which is also observed. A stimulation of the extrahepatic utilization of fat has been postulated as well as the increased liver catabolism (Greenbaum & McLean, 1953*b*). In addition to the increased rate of fat catabolism it might be expected that there would also be a reduced rate of fat synthesis. Such an inhibition of fat synthesis has already been reported by Welt & Wilhelmi (1950–51) and by Brady, Lukens & Gurin (1951). On the other hand, Allen, Medes & Weinhouse (1956) were unable to obtain a consistent inhibition and were not satisfied that growth hormone has a direct

effect on fat metabolism. Welt & Wilhelmi, using the incorporation of deuterium oxide as an index of fat synthesis, found a 50% inhibition in the rate of incorporation into both liver and carcass fat of normal rats treated with growth hormone for 24 hr. As it is already known that treatment with growth hormone causes a mobilization of body fat to the liver such that the liver fat may be doubled in 6 hr., (Weil & Ross, 1949; Greenbaum & McLean, 1953*a*), it is possible to account for the low rate of incorporation of body-water hydrogen into liver fatty acids in terms of a dilution of newly formed fat by fat mobilized from the depots. This explanation would not, however, account for the reduced rate of incorporation into carcass fatty acids, and it would therefore seem likely that the 50% reduction in deterium content observed by Welt & Wilhelmi is due to an inhibition of fatty acid synthesis. Brady *et al.* (1951) showed that hypophysectomy increased the incorporation of labelled acetate into fatty acids and, conversely, that the injection of growth hormone for 3 days into a pancreatectomized–hypophysectomized rat abolished fat synthesis entirely (as measured by incorporation of labelled acetate).

In view of these results and of the effects on fat metabolism of short-term treatments with growth hormone described by Greenbaum (1956) and Greenbaum & Graymore (1956) it was thought desirable to study the effect of relatively short-term treatment of normal rats with growth hormone on the rate of fatty acid synthesis, because it is during the first few hours of treatment that the greatest change-over of metabolic pattern occurs. Also, in addition to measurement of the rate of synthesis of fatty acids, it would be useful to find out whether there were similar effects on the synthesis of phospholipids and cholesterol. The results of the experiments described here show that whereas pituitary growth hormone greatly inhibits the synthesis of fatty acids and phospholipids it stimulates the synthesis of cholesterol.

EXPERIMENTAL

Animals. Hooded Norway rats of the Medical Research Council strain were used. The rats were 4 months old virgin females and weighed 180–220 g. They were maintained on stock diet (diet 41 of Bruce & Parkes, 1946) and allowed free access to food. Hormone-treated rats were injected intraperitoneally with 1 mg. of purified growth hormone (twice recryst. fraction A) prepared by the procedure of Wilhelmi, Fishman & Russell (1948). Controls were similarly injected with saline. One treated group was killed 6 hr. and the other 12 hr. after injection; the controls were killed 6 hr. after injection.

Preparation and incubation of tissue slices. After the allotted time interval the rats were killed by breaking their necks and the liver was rapidly excised and placed in ice-cold Ringer bicarbonate solution (Krebs & Henseleit, 1932). The liver was then sliced on a hand microtome to give 4–5 g. of slices. Samples of the residual tissue were taken for the determination of the nitrogen content and finally all remaining tissue was weighed to get the total weight of liver present. The slices were divided as follows: (*a*) Into each of three flasks similar to those described by Chernick, Masoro & Chaikoff (1950) was placed about 600 mg. of slices in 6 ml. of medium. The medium was Krebs–Ringer bicarbonate solution containing sodium [*carboxy*-^{14}C]acetate, sodium [*carboxy*-^{14}C]pyruvate or sodium [α-^{14}C]pyruvate at a concentration of 0·02 M and having a specific activity of between 5 and 10 μc/m-mole. (*b*) Into each of two wide-mouthed stoppered conical flasks was placed about 800 mg. of slices in 8 ml. of the same medium as above, for the study of phospholipid synthesis. A mixture of $O_2 + CO_2$ (95:5) was passed through both sets of flasks, which were then incubated with shaking for 3 hr. At the end of this time further treatment of groups (*a*) and (*b*) was as follows.

Carbon dioxide. This was determined and isolated (see below) from the incubates of group (*a*). CO_2-free aqueous KOH (1·5 ml. of 20%, w/v) was injected into the centre well and, after shaking for a further 5 min., 1 ml. of 4 N-HCl was tipped into the flask from the side arm. Absorption of CO_2 was allowed to continue for 2 hr. After this time the flasks were removed from the bath and the KOH collected from the centre well. The bulb and centre well were repeatedly washed with CO_2-free water and the washings added to the KOH. This solution was used to estimate the total CO_2 and its specific activity. The slices and medium in the main compartment were centrifuged. The supernatant fluid was discarded and the slices were transferred to a 100 ml. flask with the aid of 10 ml. of 4% (w/v) ethanolic KOH. After refluxing overnight this solution was used for the determination of the specific activity of the fatty acids and cholesterol as follows.

Cholesterol. The digest was transferred to a separating funnel with about 25 ml. of 50% ethanol and then extracted with light petroleum (b.p. 60–80°; 4 × 15 ml.). The combined solvent layers were evaporated to about 20 ml. and 5 ml. of a 0·2% solution of digitonin in 50% ethanol was added. The volume was then reduced to 2 ml.; 10 ml. of water was added and the whole brought to the boil. When cool, 20 ml. of acetone was added and the whole then centrifuged. The cholesterol digitonide precipitate was washed twice with acetone and twice with ether and then dried over $CaCl_2$.

Fatty acids. After removal of the unsaponified fraction the aqueous ethanolic layer was evaporated on a water bath to remove all organic solvents and then acidified. The free fatty acids were extracted with light petroleum (4 × 25 ml.). The combined solvent layers were washed with water (3 × 25 ml.), dried over Na_2SO_4 and finally evaporated to dryness.

Phospholipids. The contents of the conical flasks used in group (*b*) were centrifuged, the supernatant fluid was discarded and the slices were homogenized in 5 ml. of cold trichloroacetic acid (10%, w/v). The suspension was centrifuged and the residue re-extracted with 5 ml. of cold 10% trichloroacetic acid. The trichloroacetic acid extracts were rejected. The solid residue was extracted once with 5 ml. of warm 80% ethanol, twice with 4 ml. portions of absolute ethanol and finally once with 4 ml. of warm ether. The alcohol and ether extracts were combined and the solvents removed. The residue was extracted with light petroleum

(4 × 3 ml.) and the volume of the combined petroleum layers was reduced to about 2 ml. by distillation. Then 15 ml. of acetone and 0·5 ml. of a 4·5% (w/v) solution of $MgCl_2$ in ethanol was added and the whole allowed to stand for 30 min. The precipitated phospholipids were separated by centrifuging and dried over $CaCl_2$. In view of the difficulties involved in making a quantitative extraction and recovery of the various fractions, no attempt was made to weigh the fractions isolated.

Measurement of radioactivity

The device used for displacing the CO_2 from the bicarbonate solution is that currently used for wet combustion of organic compounds. This, together with the high-vacuum apparatus, the McLeod gauge and the methods used, have been described in detail by Glascock (1954).

Measurement of total carbon dioxide and its specific activity. The volume of solution and washings removed from the centre well of each flask was accurately measured and a 0·5 ml. portion pipetted into a tube fitted with a tap funnel and a side arm by means of which it was attached to a high-vacuum apparatus. The contents of the tube were frozen in an acetone–solid CO_2 bath and evacuated with a mercury diffusion pump. The frozen solution was melted and 0·5 ml. of 10% (v/v) H_2SO_4 run into it through the tap funnel. The CO_2 evolved was passed *in vacuo* through a U-trap cooled in an acetone–solid CO_2 bath and measured in a McLeod gauge, the trap of which was maintained at −78° to condense any trace of water vapour that may have escaped the U-trap. The volume of CO_2 so collected was usually about 300 μl. Its specific activity, which in most experiments was of the order of 4000 counts/min./ml. of CO_2, was determined by counting at least two accurately measured portions of each gas sample in a gas counter. The values for any given gas sample always agreed to within ±1·5% after counting for at least 10 000 counts. The total activity collected was calculated as the product of the total volume of solution, its CO_2 content and the specific activity of the CO_2.

Specific activity of fatty acids, phospholipids and cholesterol. In all experiments but the last two the specific activity of the carbon of the lipid fractions isolated from the slices was determined by dry combustion and counting of combustion CO_2 in a gas counter. The specific activity of the carbon of the cholesterol was calculated by multiplying that of the digitonide by the factor calculated from the carbon contents of the two compounds.

In the last two experiments the samples were counted uncombusted in a Tracerlab windowless flow counter (Tracerlab Inc., 130 High St., Boston 10, Mass, U.S.A.; Model SC 16). The cholesterol digitonide was counted at infinite thickness (50 mg./cm.²) and the phospholipid at a thickness of 5 mg./planchet (area 1·63 cm.²). This was achieved by carrying out a phosphorus determination on a solution of the phospholipid fraction and then evaporating the required volume containing 5 mg. of phospholipid to dryness on the planchet. All results are calculated as counts/mg. of carbon, but no cross-calibration of the gas- and solid-counting methods was necessary since each experiment was self-contained.

RESULTS

The extent of incorporation of the radioactive carbon from the various substrates into carbon dioxide, fatty acids, phospholipids and cholesterol by liver slices is shown in Table 1 and as histograms in Fig. 1. In Fig. 1 the specific activities of the fractions from the experimental rats have been

Table 1. *Effect of pituitary growth hormone on the rates of incorporation of the labelled carbon of pyruvate or acetate into the carbon dioxide, fatty acids, phospholipids and cholesterol in rat-liver slices*

The incubation mixtures contained the labelled substrates at a concentration of 0·02 M and having 5–10 μc/m-mole. For further details see text.

	Carbon dioxide. 10^{-5} × total activity (counts/min.)			Fatty acids. Specific activity (counts/min./mg. of C)			Phospholipids. Specific activity (counts/min./mg. of C)			Cholesterol. Specific activity (counts/min./mg. of C)		
Substrate	Control	6 hr. group	12 hr. group	Control	6 hr. group	12 hr. group	Control	6 hr. group	12 hr. group	Control	6 hr. group	12 hr. group
[*carboxy*-^{14}C]-Pyruvate	4·44	4·63	4·56	0	0	0	21	43	38	0	0	0
[α-^{14}C]Pyruvate	1·58	1·95	1·94	4 050	1950	1590	—	—	—	—	—	—
	1·01	1·20	1·43	2 840	239	117	—	—	—	—	—	—
	0·89	0·90	0·75	2 012	769	448	—	—	—	—	—	—
	1·15	0·90	0·82	1 709	669	209	1066	623	345	657	1075	422
	0·48	0·60	0·52	959	680	274	935	872	549	447	473	324
	—	—	—	—	—	—	765*	505*	298*	817*	1180*	800*
	—	—	—	—	—	—	610*	453*	356*	523*	774*	711*
[*carboxy*-^{14}C]-Acetate	2·34	—	2·37	14 450	—	1833	—	—	—	1980	—	2840
	2·34	—	2·92	14 450	—	1455	—	—	—	1980	—	2530
	1·79	1·83	—	9 967	3660	—	—	—	—	1412	1360	—
	1·79	1·77	—	9 967	6155	—	—	—	—	1412	1994	—
	2·35	—	2·07	5 010	—	817	2675	1210	1148	688	—	1131
	1·90	1·89	2·05	6 382	1240	816	—	—	—	1125	1800	800
	—	—	—	—	—	—	1335*	914*	527*	790*	1005*	887*
	—	—	—	—	—	—	1280*	624*	512*	642*	897*	813*

* These samples were measured on a windowless flow counter.

expressed as a percentage of the specific activities of those from the control rats. This method of expression has been used to avoid the difficulty of grouping results from experiments in which the initial specific activities of the substrates were not always the same. The data from any one experiment are comparable because the same medium was used for both treated and untreated rats, but the initial specific activities of the substrates varied by as much as a factor of 2 in separate experiments.

Respiratory carbon dioxide

It will be seen (Fig. 1*a*) that treatment of the rats with growth hormone had little or no effect on the relative rates of production of carbon dioxide from any of the three substrates. The total activity of the carbon dioxide from [*carboxy*-^{14}C]pyruvate ($4{\cdot}44 \times 10^5$ counts/min.) was roughly four times that produced from [α-^{14}C]pyruvate and twice that from [*carboxy*-^{14}C]acetate (Table 1).

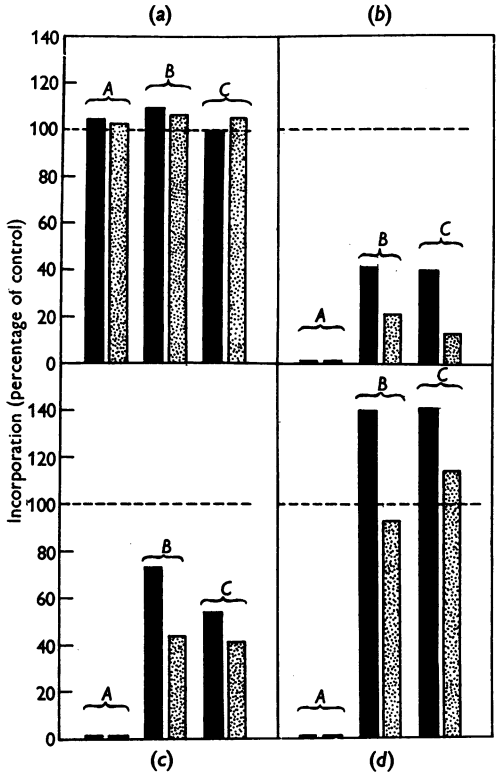

Fig. 1. Incorporation of ^{14}C from labelled pyruvate or acetate into (*a*) the CO_2, (*b*) fatty acids, (*c*) phospholipids and (*d*) cholesterol of rats treated with growth hormone. The results are expressed as percentages of the control specific activity. The columns represent values for rats treated with growth hormone for 6 hr. (solid columns) and for 12 hr. (dotted columns). *A*, [*carboxy*-^{14}C]Pyruvate; *B*, [α-^{14}C]pyruvate; *C*, [*carboxy*-^{14}C]acetate.

Fatty acids

The incorporation of substrate carbon into fatty acids is shown in Fig. 1*b*. None of the carboxyl carbon of pyruvate was found in the fatty acids. The rate of incorporation of the carbonyl carbon was greatly reduced in the rats treated with growth hormone, the effect being more pronounced after 12 hr. than after 6 hr. The mean specific activity of the controls was 2320/counts/min./mg. of carbon, and this was reduced to 41 % of the control level 6 hr. after growth-hormone treatment and to 21 % after 12 hr. Incorporation of the carboxyl carbon of acetate was also reduced by previous treatment of the rats with growth hormone, and again the effect was most marked after 12 hr. treatment, the levels at 6 and 12 hr. being 39 and 13 % of the control levels respectively. The rate of incorporation of ^{14}C from [α-^{14}C]pyruvate or [*carboxy*-^{14}C]acetate was inhibited in all experiments, and the diminution in rate was statistically significant for both substrates at both times in all experiments. [All values were significant with $P < 0{\cdot}05$ (Fisher, 1944).]

Phospholipids

No incorporation of the carboxyl carbon of [*carboxy*-^{14}C]pyruvate into phospholipids was found in either normal or treated animals. The incorporation of the labelled carbon of [α-^{14}C]pyruvate was inhibited by growth hormone, but to a less extent than into fatty acids. The same was true for [*carboxy*-^{14}C]acetate.

Cholesterol

The carboxyl carbon of pyruvate was not incorporated into cholesterol. The carbonyl carbon atom, however, was incorporated more rapidly into the liver cholesterol of rats treated with growth hormone than into the liver cholesterol of control rats. The specific activity of the cholesterol from rats injected 6 hr. before being killed was 140 % of that of the control. By 12 hr. the incorporation was no longer stimulated and the 12 hr. value is indistinguishable from that of the controls. A similar pattern was obtained with [*carboxy*-^{14}C]acetate, a stimulation at 6 hr. and a return towards normal values by 12 hr.

DISCUSSION

The results given above show that the livers of rats previously injected with pituitary growth hormone have a greatly reduced ability to synthesize fatty acids from either pyruvate or acetate. In general the growth-hormone treatment in many cases reduced the rate of incorporation of the labelled carbon into fatty acids from these substrates to between one-tenth and one-fifth of that found in the livers from untreated rats. The inhibitory effect is

greater at 12 than at 6 hr. and there is no evidence whether the maximum effect is obtained at 12 hr. or later. But in the twenty-five growth-hormone-treated rats used in the 6 and 12 hr. groups an inhibition was always observed. This result is in agreement with the previous observations of Welt & Wilhelmi (1950–51), of Brady *et al.* (1951) and others. Only Allen *et al.* (1956) failed to obtain such an inhibition. Of the five experiments reported by these last authors an inhibition was observed in four and a stimulation in one. Their maximum inhibition, obtained after injecting 15 mg. of growth hormone as a single dose after a 3-week treatment with daily injections of 0·5 mg., was only to 38 % of control level. In the experiments reported here a single injection of 1 mg. had a greater effect than the protracted treatment described by Allen *et al.* (1956) and on several occasions gave incorporation rates of less than 10 % of the control values. Their variable and uncertain results led Allen *et al.* to conclude that the effect of growth hormone in diminishing the rate of fatty acid synthesis was not established. In view of the results given above it seems quite clear that the injection of the hormone does, in fact, lead to a lowered rate of fatty acid synthesis. A possible explanation of the results of Allen *et al.* lies in the unusual condition applied to their rats immediately before being killed. The animals were first starved for 24 hr. and then fed again for 24 hr. The purpose of this procedure was not stated but, whatever the reason, this procedure would tend to introduce some variability and also make the results not comparable with those of other workers using more orthodox methods.

It is not possible to say from the results of the present work that growth hormone has a direct action on fatty acid synthesis. Other hormones, notably those of the pancreas and adrenals, also affect it (Welt & Wilhelmi, 1950–51; Brady *et al.* 1951), and it would have to be shown that the growth hormone used was entirely free of corticotrophic, pancreatrophic or thyrotrophic hormone. The preparation used (fraction A of Wilhelmi *et al.* 1948) is virtually free from these contaminants but possibly not entirely so. Nevertheless, in view of the very small quantity of such contaminants which could have been present in the 1 mg. of growth hormone injected, it is probable that the prime agent in the inhibition of fatty acid synthesis observed in these experiments was growth hormone itself.

The very low incorporation rate in the treated animals could be due to several causes. First, it could be an artifact, the incorporation rate being identical in the treated and control groups, but the mobilization of fat to the liver after growth-hormone treatment (Szego & White, 1949; Greenbaum & McLean, 1953*a*) causing dilution of the newly formed fat to give low specific activities. Secondly, there could be a reduction in the rate of formation of the C_2 fragments, although the activity of the enzymes synthesizing fatty acids remains unchanged; thirdly, there could be an increased rate of formation of C_2 fragments from endogenous sources which dilute those arising from the substrate; finally, there is the possibility of a reduction in the activity of the fatty acid-synthesizing mechanisms.

It is unlikely that dilution of freshly formed fatty acids by fatty acid mobilized from the depots could account for the low specific activities in treated animals. Greenbaum & McLean (1953*a*) found only a doubling of the liver fat as a result of the injection of growth hormone, and similar increases were found by Li, Simpson & Evans (1949), Szego & White (1949) and Weil & Ross (1949). In order to account for the low specific activity of the fatty acids in the livers of the treated rats the endogenous level of fat would have to be increased by a factor of 4 or 5, or even 20, which is far greater than anything previously reported.

It also seems unlikely that the low specific activity found could be due to a decreased rate of formation of labelled acetyl-coenzyme A since the rates of production of $^{14}CO_2$ from the carboxyl group of pyruvate is virtually identical in normal and growth-hormone-treated animals.

Dilution of the labelled acetyl-coenzyme A before its synthesis into fatty acids is also unlikely. Glycolysis is not stimulated by growth hormone (Recant, 1952), so that this pathway probably does not contribute extra acetyl-coenzyme A; nor is protein catabolism a likely source since protein catabolism is decreased and protein synthesis increased in growth-hormone-treated rats (Russell & Cappiello, 1949; Hoberman, 1949–50). Thus the low rate of incorporation found probably reflects a genuine reduction in the rate of fatty acid synthesis.

The interpretation of these results in terms of events occurring in the whole animal is difficult. One of the main characteristics of a growth-hormone-treated rat is that it loses fat as it grows (Young, 1945; Greenbaum, 1953). It might be expected, therefore, that a growth-hormone-treated rat would show both an increased rate of fatty acid degradation and a decreased rate of fatty acid synthesis. Measurements of the rate of fatty acid oxidation (Greenbaum & McLean, 1953*b*; Allen *et al.* 1956) show that after 2 days of treatment the rate of fatty acid oxidation in the livers of growth-hormone-treated rats is the same as in controls. If the liver really reflects the rate of fat oxidation in the body as a whole it would appear that the total decrease of body fat in the treated rats is achieved by a reduced rate of

fat synthesis rather than by an increased rate of oxidation.

In our experiments the phospholipid fraction also shows a reduced rate of incorporation of labelled carbon from [α-^{14}C]pyruvate and from [*carboxy*-^{14}C]acetate in treated rats. The decreases are less pronounced in phospholipid synthesis than in fatty acid synthesis. This finding is somewhat surprising in that growth hormone causes a reduction in the amount of fatty acid carbon incorporated into the phospholipids at the same time as it causes an increase in the rate of phosphorus incorporation (Greenbaum, Graymore & Slater, 1957). This difference is difficult to explain unless it is supposed that different parts of the phospholipid molecule have different rates of turnover.

Growth hormone causes an increase in the rate of incorporation of acetate and pyruvate carbon into cholesterol and not a decrease. With [α-^{14}C]pyruvate the rate is increased by 40% 6 hr. after hormone treatment but returns to normal by 12 hr. When [*carboxy*-^{14}C]acetate is the substrate the increase at 6 hr. is 33%, and although the level returns towards that of the control by 12 hr. it is still higher than in the control animals. No explanation for this difference is available. The stimulation of cholesterol synthesis simultaneously with a decrease in fatty acid synthesis is in keeping with the results of Allen *et al.* (1956). An inverse relationship of this kind has also been found by Hotta & Chaikoff (1952) in the livers of diabetic rats. Presumably fatty acid and cholesterol synthesis compete for the same precursor, and cholesterol synthesis therefore increases when the competition is reduced by a reduction in the rate of fatty acid synthesis.

SUMMARY

1. The effect of administration of pituitary growth hormone on the rates of incorporation of labelled carbon from pyruvate and acetate into carbon dioxide, fatty acids, phospholipids and cholesterol by rat-liver slices has been investigated.

2. There was no difference in the rate of production of carbon dioxide from any of these substrates in normal or treated rats. The labelled carbon of [*carboxy*-^{14}C]pyruvate was only found in the carbon dioxide.

3. Growth hormone reduced the rate of incorporation of labelled carbon from [α-^{14}C]pyruvate and [*carboxy*-^{14}C]acetate into both fatty acids and phospholipids.

4. The rate of incorporation of labelled carbon into cholesterol appeared to be stimulated by growth hormone.

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