insoluble cellulose to soluble products by enzymes from *Myrothecium verrucaria*.

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The Effect of Hypophysectomy of the Rat and of Treatment with Growth Hormone on the Incorporation *in vivo* of Radioactive Amino Acids into the Proteins of Subcellular Fractions of Rat Liver

By A. KORNER

Department of Biochemistry, University of Cambridge

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The liver of the rat, like the body as a whole, decreases in size and protein content as a result of hypophysectomy, and this fall can be prevented to a large extent by treatment of the rat with growth hormone (Simpson, Evans & Li, 1949). There is some evidence that growth hormone stimulates growth by increasing the rate of protein biosynthesis (Russell, 1955) rather than by slowing down the rate of catabolism of proteins.

Zamecnik & Keller (1954) have described the preparation of a cell-free system from rat liver which brings about the incorporation of radioactive amino acids into protein *in vitro*. In the soluble fraction of the cell the amino acids are prepared for incorporation into proteins by amino acid-activating enzymes (Hoagland, Keller & Zamecnik, 1956) and by forming an amino acid-soluble ribonucleic acid intermediate (Hoagland, Zamecnik & Stephenson, 1957). In this system the assembly of the polypeptide chains occurs in the ribonucleo-protein microsomal particles of the endoplasmic reticulum (Littlefield, Keller, Gross & Zamecnik, 1955).

With a system of the type described by Zamecnik & Keller (1954), Korner (1958, 1959) has shown that the microsomes from the livers of hypophysectomized rats are significantly less able than those from livers of normal rats to assemble amino acids into proteins. Hypophysectomy of the rat did not result in any change, that was detectable by the system used *in vitro*, in the ability of the soluble fraction of the liver cells to prepare amino acids for incorporation. Treatment of hypophysectomized rats with growth hormone restored, to some extent,

the diminished ability of the liver microsomes to incorporate amino acids into protein. Treatment of normal rats with growth hormone enhanced the ability of liver microsomes to incorporate amino acids into protein.

The experiments described in this paper were carried out to see how far these findings *in vitro* could be confirmed and extended by experiments on the incorporation of amino acids into proteins in whole animals.

METHODS

Animals. Young, adult, female albino rats, of the same age and body weight, were selected from an inbred strain and were placed, at random, into two groups. The rats of one group were hypophysectomized under ether anaesthesia by the parapharyngeal approach (Ingle & Griffith, 1942), and the rats of the second group were submitted to a sham operation. After recovery from the operation each rat was housed in an individual cage and all rats were given the same ration of powdered diet (diet 41, Bruce & Parkes, 1949) made into a thick paste with water. The daily ration, established by trial and error, was the largest quantity of food which was entirely consumed by every rat (approx. 10 g./100 g. of rat/day). Water was freely available. The animals were kept on this ration for 3 weeks before being used in an experiment. The rats were not starved during the night before they were killed although each rat had eaten its last ration several hours before it was killed.

Growth hormone. Ox growth hormone, prepared by the method of Wilhelmi, Fishman & Russell (1948), was used. It was dissolved in 0.9% NaCl which had been adjusted to pH 7.5–8.0 with 0.1N-NaOH. The dose was injected subcutaneously in 0.5 ml. of 0.9% NaCl and the control rats were given 0.5 ml. of 0.9% NaCl by subcutaneous injection.

Radioactive compounds. Generally (G) labelled L-[G-¹⁴C]-leucine, DL-[1-¹⁴C]leucine and hydrolysed algal protein

labelled with ^{14}C were used and were obtained from The Radiochemical Centre, Amersham, Bucks. Their specific activities were 66.9, 40.6 and 88 $\mu\text{C}/\text{mg}$. respectively. They were dissolved in such a volume of 0.9% NaCl at pH 7.0 that the appropriate dose (between 10 and 20 $\mu\text{C}/\text{rat}$) could be administered in a volume of 0.5 ml. Every rat in an experiment was given the same amount of radioactivity and no attempt was made to correlate the dose administered to a rat with its body wt. The body weights of the rats used were between 145 and 160 g. 1 μC of [^{14}C]amino acid would record about 6.5×10^4 counts/min. on the Geiger-Müller end-window (1.5–2.5 mg./cm.²) counter used.

Experimental procedure. The rats were anaesthetized with Nembutal and the femoral vein was exposed. The dose of radioactive amino acid was injected into the vein in 0.5 ml. of 0.9% NaCl. A small incision was made in the abdominal wall above the liver and gentle pressure on the sides of the abdomen forced the first lobe of the liver into the incision. The blood vessels leading to this lobe were tied off and the lobe was removed. Two types of experiment were carried out. In one, the specific activity of the unseparated liver proteins of each lobe was determined, together with the specific activity of the free intracellular amino acids, and in the other, the liver tissue was separated into subcellular components and the specific activity of the proteins of each was determined. In the first type of experiment each lobe of liver as it was removed from the rat was plunged into boiling water, cut up with scissors and boiled. In the second type of experiment each liver lobe was placed in ice-cold 0.35 M-sucrose containing an excess of unlabelled amino acids corresponding to the radioactive ones injected. The liver was cut into small pieces with scissors. The exact time interval, from the end of the injection of the radioactive amino acids until the moment the liver was placed in the boiling water or in the cold sucrose, was noted (2–3 min.). The second lobe of the liver was removed in a similar manner 8–9 min. after the injection of the amino acids and the remainder of the liver was removed 20 min. after the injection, and each lobe was again placed in boiling water or in ice-cold sucrose, according to the experiment being carried out. Just before the last lobe of liver was removed, a sample of blood was taken by cardiac puncture with an oxalate-treated hypodermic syringe and the blood was added to boiling water. The blood proteins were precipitated and washed in the manner described below for liver tissue and the combined washings were used to determine the specific activity of the free amino acids in blood.

Determination of total protein specific activity and intracellular amino acid specific activity. The boiled liver was thoroughly homogenized in a tight-fitting, motor-driven, glass homogenizer and the brei washed with water into the centrifuge tube in which it had been boiled. An equal volume of 0.5 N-perchloric acid was added to the brei to ensure precipitation of all the protein. The precipitated protein was centrifuged at 600 g. The supernatant fluid was removed and the protein precipitate was resuspended by homogenization, once in water and once in 0.25 N-perchloric acid and the precipitated protein spun down each time by centrifuging. These washings were combined with the original supernatant fluid and were used to determine the specific activity of the free intracellular amino acids, and the protein was prepared for counting in the way described below.

Triplicate 0.5 ml. samples of the protein-free washings of the protein of each tissue sample were pipetted on to disks of lens paper on lacquered aluminium planchets and 0.1 ml. of a dilute polyvinyl alcohol soln. was added to ensure even spreading. The sample was dried under an infrared lamp and the radioactivity determined in a Geiger-Müller windowless, gasflow counter. Enough counts were recorded to reduce the standard error to 3% or less and a correction was applied for the background counts. Three further 0.5 ml. samples of the combined washings were used for the determination of the amino acid content of the washings by a modification of the ninhydrin method of Moore & Stein (1948). Each sample was diluted with water to 2 ml. and 0.2 ml. of a 10% solution of recrystallized ninhydrin in 2-methoxyethanol was added. A sample (1 ml.) of a freshly prepared mixture consisting of 2 ml. of 0.01 M-KCN, 23 ml. of 8.3 M-sodium acetate–1.66 M-acetic acid and 75 ml. of 2-methoxyethanol was added and the tubes were capped and boiled for 20 min. After boiling, the tubes were cooled in running water for 5 min. and 6.3 ml. of 60% ethanol was added and the mixture shaken vigorously for 30 sec.; the absorption at 570 m μ was read in the Beckman spectrophotometer. The α -amino N content (μg .) of the sample was calculated from a standard curve constructed for valine. The counts/min./ μg . of α -amino N was calculated. In experiments where hydrolysed protein labelled with ^{14}C was used as the source of radioactivity, this method gives an estimate of the specific activity of the intracellular free amino acids which is of fair accuracy. There is, however, an assumption that the radioactivity measured is all present in the free amino acids. It is possible that small peptides may be present and may be radioactive. However, when samples were hydrolysed by treatment with 6 N-HCl at 100° for 24 hr. before determining the amino acid specific activity, no significant alteration in specific activity was found, compared with that of unhydrolysed samples. Radioactive nucleotides may also be present in the sample, but any differences which may exist between the specific activities of free intracellular nucleotides in differently treated rats was considered to be small enough to be neglected. Similarly, the presence in the sample of amino acids which were not present in the mixture of injected radioactive amino acids was ignored.

Separation of cell fractions. When each sample of liver had been cooled, it was removed into fresh, cold 0.35 M-sucrose and homogenized with a hand-operated homogenizer kept cold in an ice bath. Nuclei, mitochondria, microsomes and the soluble fraction of the cell were separated by differential centrifuging of the homogenate by a modification of the procedure described by Hogeboom, Schneider & Palade (1948). The tissue brei was centrifuged at 15 000 g for 10 min. at 0° to precipitate nuclei, mitochondria and cell debris. The supernatant fluid was carefully removed from the pellet and microsomes and the soluble fraction of the cell were separated from it in the way described below.

The pellet from the centrifuging at 15 000 g was resuspended by homogenization in cold 0.35 M-sucrose and the brei was centrifuged at 800 g for 10 min. at 0°, when the nuclei and cell debris were spun down. The supernatant fluid was carefully removed and mitochondria were separated from it (see below). The pellet containing nuclei and cell debris was suspended in cold 0.35 M-sucrose and centrifuged at 200 g to remove cell debris. The supernatant fluid was recentrifuged at 200 g and then at 800 g to spin down

the nuclei. The nuclei were washed twice by resuspension in 0.35 M-sucrose followed by centrifuging at 800 g. They were then suspended in water and their proteins precipitated with cold 0.5 N-perchloric acid containing unlabelled amino acids.

The supernatant fluid from the first centrifuging at 800 g was centrifuged again at 800 g for 10 min. The supernatant fluid was removed and centrifuged at 8000 g for 10 min. at 0°, when mitochondria were spun down. The supernatant fluid was discarded. The mitochondrial pellet so obtained was washed twice by resuspension in cold 0.35 M-sucrose, followed by centrifuging at 6000 g for 10 min. and then the mitochondria were suspended in water and their proteins precipitated with cold 0.5 N-perchloric acid containing unlabelled amino acids.

The supernatant fluid from the first 15 000 g centrifuging was centrifuged at 105 000 g for 1 hr. at 0° in a Spinco model L preparative ultracentrifuge. The supernatant fluid was removed from the microsome pellet and the proteins of the soluble fraction of the cell were precipitated with cold 0.5 N-perchloric acid containing unlabelled amino acid. The microsome pellet and the sides of the tube were carefully rinsed with ice-cold 0.35 M-sucrose and the tube was wiped with tissue paper. The microsome pellet was treated in one of two ways. Either it was suspended in water and its proteins were precipitated with cold 0.5 N-perchloric acid, or the microsome pellet was suspended by homogenization in a hand-operated homogenizer in 2 ml. of cold 0.2 M-glycylglycine buffer, pH 8.0, and treated with cold, freshly prepared 5% sodium deoxycholate soln. in 0.2 M-glycylglycine buffer, pH 8.0. The pH was determined with a glass-electrode pH meter standardized against a standard buffer soln. made in accordance with the manufacturer's instructions from Burroughs Wellcome and Co. N.B.S. buffer solution tablets. The pH of the standard soln. was 6.99 at 25°. The volume of sodium deoxycholate soln. added was that which was calculated to contain 0.75 mg. of sodium deoxycholate/mg. of microsome protein. The suspension was kept in ice for 10 min. and a volume of ice-cold water was then added to give a deoxycholate concentration of 0.5% and the suspension was centrifuged at 105 000 g for 2 hr. It has been shown (Littlefield *et al.* 1955; Palade & Siekevitz, 1956) that treatment of microsome pellet in this fashion renders soluble much of the protein of the microsomes, leaving the ribonucleoprotein particles of the endoplasmic reticulum undissolved. After the centrifuging, the supernatant fluid of protein soluble in deoxycholate was carefully removed from the pellet of ribonucleoprotein microsome particles and its proteins were precipitated

with cold 0.5 N-perchloric acid containing unlabelled amino acids. The pellet of microsome particles was suspended by homogenization in water and its proteins were precipitated with 0.5 N-perchloric acid in a similar fashion. The ribonucleic acid/protein ratio of the pellet of microsome particles was determined and served as a guide to the degree of separation of the ribonucleoprotein particles from the rest of the endoplasmic reticulum. This ratio was between 0.65 and 0.85.

Preparation of the radioactive protein for counting. The precipitated proteins were washed, lipid and ribonucleic acid (RNA) were extracted and the protein was dried, plated and its radioactivity determined by counting in a Geiger-Müller end-window counter (1.5–2.5 mg./cm.²) in the way previously described (Korner, 1959). The results are given as counts/min./mg. of protein after corrections have been made for self-absorption and background counts.

Determination of ribonucleic acid. The RNA content of samples of the liver fractions was determined by a slight modification of the method of Scott, Fraccastoro & Taft (1956) as previously described (Korner, 1959).

Determination of protein content. The N content of samples of the cell fractions was determined by the micro-Kjeldahl technique and a correction applied for the N content of any RNA present. The corrected value for N was used to calculate the protein content of the samples by using the factor 6.25.

Statistical treatment of results. At least four rats were used for each experimental procedure and the experimental observations were used to calculate the mean value and the standard error of the mean (S.E.M.). Student's *t* test was applied to ascertain if differences between mean values were statistically significant, and values of Fisher's *P* are given where differences between means are considered to be significant.

RESULTS

Table 1 shows the mean specific activities of the unseparated liver proteins of each of the three lobes of liver of normal rats, of hypophysectomized rats and of hypophysectomized rats given 0.3 mg. of growth hormone/day for 5 days. The lobes were removed 2, 8 and 20 min. after injection of 20 µC of hydrolysed [¹⁴C]protein. Hypophysectomy has caused a statistically significant reduction in the incorporation of the radioactive amino acids into protein and treatment of the hypophysectomized rats with growth hormone has resulted in more

Table 1. *Effect of hypophysectomy of the rat, and of treatment of hypophysectomized rats with 0.3 mg. of growth hormone/day for 5 days, on the incorporation of intravenously injected algal protein hydrolysate labelled with ¹⁴C (20 µC) into the proteins of the three lobes of liver*

Lobe of liver	Time lobe removed after injection of amino acids (min.)	Specific activity of protein (mean ± S.E.M.; four rats in each group)			Fisher's <i>P</i> less than	
		Normal rats (N)	Hypophysectomized rats (H)	Growth hormone-treated hypophysectomized rats (HG)	N, H	H, HG
1	2	62 ± 5.2	41 ± 4.1	53 ± 3.2	0.01	0.05
2	8	101 ± 7.6	64 ± 5.7	86 ± 5.9	0.01	0.02
3	20	123 ± 9.3	82 ± 6.9	103 ± 6.8	0.01	0.05

Table 2. *Effect of hypophysectomy of the rat, and of treatment of hypophysectomized rats with 0.3 mg. of growth hormone/day for 5 days, on the specific activity of the free amino acids in liver and blood, after intravenous injection of hydrolysed algal protein labelled with ¹⁴C (20 μC)*

Lobe of liver	Time lobe removed after injection of amino acids (min.)	Counts/min./μg. of α-amino N (mean ± S.E.M.; four rats in each group)		
		Normal rats	Hypophysectomized rats	Growth hormone-treated hypophysectomized rats
1	2	46 ± 6.3	41 ± 4.3	44 ± 5.1
2	8	29 ± 4.6	39 ± 5.2	36 ± 4.1
3	20	32 ± 4.1	38 ± 4.2	31 ± 3.9
Blood	19	94 ± 17.3	84 ± 16.2	99 ± 19.5

Table 3. *Effect of hypophysectomy of the rat, and of treatment of hypophysectomized rats with 0.4 mg. of growth hormone/day for 6 days, on the incorporation of intravenously injected DL-[1-¹⁴C]leucine (15 μC) into the proteins of the nuclei, mitochondria, microsomes and soluble fraction of the three lobes of liver*

Lobe 1 was removed 2 min., lobe 2, 8 min. and lobe 3, 20 min. after injection of the amino acid.

Type of rat	Lobe of liver	Protein specific activity (mean ± S.E.M.; six rats in each group)			
		Nuclei	Mitochondria	Microsomes	Soluble fraction
Normal	1	47 ± 4.1	49 ± 3.8	275 ± 17.9	30 ± 2.6
	2	55 ± 4.7	73 ± 5.1	430 ± 51.1	59 ± 3.9
	3	77 ± 4.9	118 ± 9.2	312 ± 36.2	81 ± 6.9
Hypophysectomized	1	34 ± 3.1	29 ± 1.9	189 ± 19.5	19 ± 1.6
	2	46 ± 3.9	55 ± 3.2	240 ± 27.6	33 ± 5.0
	3	61 ± 4.2	82 ± 6.2	285 ± 22.0	39 ± 3.2
Growth hormone-treated hypophysectomized	1	39 ± 2.9	35 ± 3.4	235 ± 24.5	22 ± 2.9
	2	50 ± 4.1	63 ± 5.1	336 ± 36.3	52 ± 4.6
	3	66 ± 4.6	99 ± 8.3	316 ± 36.6	68 ± 7.1

amino acid being incorporated into liver protein than was the case with the untreated hypophysectomized rats. The increased uptake resulting from treatment with growth hormone is, at best, only probably significant statistically.

Table 2 records the specific activities of the free intracellular amino acids and it can be seen that no significant differences were found between the three groups. The scatter of the individual readings was large but it would appear that, whereas the specific activity of the intracellular free amino acids of the livers of normal rats and of hypophysectomized rats treated with growth hormone fell from an initial high level, that of the hypophysectomized rats tended to maintain a fairly constant level for the experimental period of 20 min. Little difference was apparent between the specific activities of the free amino acids of serum in the three groups of animals.

When the liver lobes were divided into the four main subcellular fractions of nuclei, mitochondria, microsomes and soluble fraction of the cell, it became clear that the specific activities of the proteins of these subcellular fractions from hypophysectomized rats were all significantly less than those of the corresponding fractions from normal

rats (Table 3). The diminished incorporation of amino acids into the proteins of liver fractions from hypophysectomized rats is particularly marked in the microsomes and the soluble fraction of the cell, but it is apparent, albeit to a lesser extent, in the other two cell components. Here again, treatment of the hypophysectomized rats with growth hormone restored, to a large extent, the ability of the liver to incorporate amino acids into the proteins of each of the four cell fractions but treatment of hypophysectomized rats with growth hormone failed to cause complete restoration of the ability to incorporate amino acids into protein to the extent shown by normal rats.

When the microsome pellet was divided into microsomal particles and material soluble in deoxycholate, it was found (Fig. 1) that a very rapid rise in protein specific activity occurred in the microsomal particles of normal rats, reaching a maximum value within 2 min. The deoxycholate-soluble fraction showed a slower rise in specific activity, but the rise in specific activity continues for much longer than was the case with the microsomal particles. The specific activity of the proteins of the soluble fraction of the cell rises at the slowest rate. These results are similar to those

reported by Littlefield *et al.* (1955) except that these workers showed that the specific activity of the protein of the microsomal particles fell below that of the deoxycholate-soluble material about 12 min. after the injection of a small dose of radioactive amino acid. This 'cross over' of the specific-activity curves was not found in the experiments reported here, in which somewhat larger doses of radioactive amino acids were used (see Discussion).

These results are consistent with the idea that the ribonucleoprotein of the microsomal particles acts as a template on which peptide chains are formed from acyl-activated amino acids. Once formed, the peptide chain peels off the template and is passed into that part of the microsome soluble in deoxycholate and thence into the soluble fraction of the cell (Littlefield *et al.* 1955).

The specific activities of the proteins of the microsomal particles, of the microsomal material soluble in deoxycholate and of the soluble fraction of the cell from the livers of hypophysectomized

rats are also shown in Fig. 1. It can be seen that the same relationship occurred between the specific activities of the proteins of the three subcellular fractions of the liver cell of hypophysectomized rats as between the same fractions from normal rat liver. It is clear, however, that the specific activity of the protein of the microsomal particles from livers of hypophysectomized rats is much less than that of the protein of these particles prepared from livers of normal rats for at least 10 min. after the injection of the radioactive amino acids. The specific activity of the protein of microsomal particles from normal rat liver falls more rapidly with time than does the specific activity of the proteins of the microsomal particles of livers of hypophysectomized rats, so that 20 min. after the injection of radioactive amino acids there is little difference between the specific activities of the proteins of the microsomal particles from the livers of differently treated rats.

Another difference between the normal and hypophysectomized rats, which is apparent from Fig. 1, is that the specific activity of the proteins of that portion of the microsomes which is soluble in deoxycholate increases at a much more rapid rate in normal rats than in rats which have been hypophysectomized. The specific activity of the proteins of the soluble fraction of the cell is also less in the hypophysectomized rats than in the normal ones (cf. Table 3).

Treatment of hypophysectomized rats with growth hormone tends to restore the extent of amino acid incorporation into protein towards that shown by normal rats and the distribution of radioactive protein between the microsomal particles, the deoxycholate-soluble material and the soluble fraction of the cell approaches that found with normal rats. Fig. 2 shows that the proteins of the microsomal particles have a greater specific activity in those hypophysectomized rats which were treated with growth hormone and that there is a more rapid fall in the specific activity of the proteins of these particles than in those of untreated hypophysectomized rats. At the same time the specific activity of the proteins of the deoxycholate-soluble portion of the microsomal particles rises more rapidly in the rats treated with growth hormone and so does the specific activity of the soluble-fraction protein.

It might appear from Fig. 1 that the specific activity of the proteins of the microsomal particles of livers from hypophysectomized rats does not reach its maximum value until about 10 min. after the injection of radioactive amino acids. An alternative explanation is that the maximum specific activity of microsomal-particle protein is reached only a little later in the hypophysectomized rats than in the normal or growth hormone-treated

Fig. 1. Effect of hypophysectomy of the rat on the incorporation of intravenously injected L-[G-¹⁴C]leucine into the proteins of the microsomal particles (●, ○), the proteins of that portion of the microsomes which is soluble in sodium deoxycholate (■, □) and the proteins of the soluble fraction of the liver cells (▲, △). Each point represents the mean of four experiments. Results from normal rats are indicated by filled-in points and those from hypophysectomized rats by open points.

hypophysectomized rats, but that it occurs after the first lobe of liver is removed from the hypophysectomized rats whereas the maximum specific activity is reached before the first lobe is removed from normal or growth hormone-treated hypophysectomized rats. Fig. 3 illustrates the results of an experiment which support this suggestion. In this experiment the first lobe of the liver was not removed from the hypophysectomized rat until 5 min. after the injection of radioactive amino acids, whereas, in contrast, the first lobe of the liver of the normal rat and of the hypophysectomized rat treated with growth hormone were removed as early as possible after the radioactive amino acid has been injected (0.7–1.2 min.). Here the maximum specific activity of the microsomal particle proteins has been reached by the time the first liver lobe was removed in the hypophysectomized rat but not in the normal rat nor the hypophysectomized rat which had been treated with growth hormone. In these experiments, the slow decline of the specific activity of the microsomal-particle protein from hypophysectomized rat liver was still apparent, as was the slow rise in the specific activity of the protein of the material soluble in deoxycholate.

It appears from Figs. 1 and 2 that hypophysectomy has caused a greater diminution of the

specific activity of the protein of the deoxycholate-soluble fraction of the microsomes than can be accounted for solely on the grounds of the smaller specific activity of the proteins of the microsomal particles in these animals. In order to see if hypophysectomy and growth hormone treatment had, indeed, altered the ability of the synthesized polypeptides to peel off the microsomal template and be transferred to the lipoprotein part of the microsomes, the data of the experiments described in Figs. 1 and 2 were used to calculate the percentage of the total counts in the undivided microsomal protein which occurs in the proteins of the deoxycholate-soluble portion of the microsome (Table 4). The only differences which emerged occurred 20 min. after the injection of radioactive amino acids, when the percentage of the counts which appeared in the deoxycholate-soluble fraction from hypophysectomized rats was less than that in the same fraction from normal rats, or from hypophysectomized rats which had received growth hormone.

Fig. 2. Effect of treatment of hypophysectomized rats with 0.2 mg. of growth hormone/day for 7 days on the incorporation of intravenously injected L-[G-¹⁴C]leucine into the protein of the microsomal particles (●, ○), the protein of that portion of the microsomes which is soluble in sodium deoxycholate (■, □) and the proteins of the soluble fraction of the liver cells (▲, △). Each point represents the mean of four experiments. Open points are used for results from hypophysectomized rats and filled-in points for hypophysectomized rats treated with growth hormone.

Fig. 3. Effect of hypophysectomy of the rat, and of treatment of hypophysectomized rats with 0.3 mg. of growth hormone/day for 5 days on the incorporation of intravenously injected DL-[1-¹⁴C]leucine into the proteins of the microsomal particles of rat liver (●, ○) and into the deoxycholate-soluble fraction of the microsomes (■, □). In these experiments the first liver lobe of the normal rat and of the hypophysectomized rat given growth hormone were removed as soon as possible after injection of the amino acid, whereas the first lobe of the liver of the hypophysectomized rat was removed 5 min. after the injection. Results from normal rats are indicated by open points, and those from hypophysectomized rats by filled-in points. Results from hypophysectomized rats treated with growth hormone are indicated by open points and broken lines.

Table 4. *Effect of hypophysectomy of the rat, and of treatment of hypophysectomized rats with 0.2 mg. of growth hormone/day for 7 days, on the percentage of the total counts in the protein of the microsomes which occurs in the deoxycholate-soluble fraction of the microsomes, after intravenous injection of L-[G-¹⁴C]leucine (15 µc)*

Data from the experiments described in Figs. 1 and 2.

Liver lobe	Time removed after injection of amino acid (min.)	Percentage of total counts/min. in microsome protein which occurs in the protein of the material soluble in deoxycholate (mean values; four rats in each group)		
		Normal rats	Hypophy- sectomized rats	Growth hormone- treated hypophy- sectomized rats
1	2	44	46	49
2	9-11	57	58	63
3	20	79	63	79

DISCUSSION

Growth hormone and protein synthesis. Experiments with cell-free preparations of rat liver have shown that amino acid incorporation into protein occurs in microsomes (Littlefield *et al.* 1955) and mitochondria (McLean, Cohn, Brandt & Simpson, 1958), and experiments with other tissues have shown that the nuclei are also capable of some protein biosynthesis (Allfrey, Mirsky & Osawa, 1957). The experiments reported in Tables 1 and 3 of this paper show that hypophysectomy inhibits, and treatment of hypophysectomized rats with various doses of growth hormone stimulates, protein synthesis in the liver *in vivo* overall, and in each of the particulate fractions of the liver cell. The experiments were short-term ones and it is highly unlikely that growth hormone can be acting solely by diminishing the amount of protein catabolism. These results lend support to the idea that the ability of growth hormone to enhance the rate of protein synthesis (see Russell, 1955) is responsible for much of the decrease in weight and protein content of rat liver which follows hypophysectomy, and the reversal of this trend in hypophysectomized animals treated with growth hormone (Simpson *et al.* 1949).

Few experiments have been reported on the influence of hypophysectomy, or of treatment with growth hormone, on the incorporation of radioactive amino acids into proteins, in the rat. Friedberg & Greenberg (1947) found that growth hormone increased the incorporation of [³⁵S]-methionine into muscle protein of hypophysectomized rats but that liver showed a reduction in amino acid uptake under the influence of growth hormone. They give no experimental evidence for this statement in the communication but cite as reference a thesis of the University of California which is not available to the author. Their experiments are open to the technical objection that

methionine can be bound to protein by non-peptide linkages unless special precautions are taken in washing the protein while preparing it for radioactive counting (Tarver, 1954).

Lee & Williams (1952) studied the effect of growth hormone, administered to normal rats, on the incorporation of [³⁵S]cystine into nuclear, mitochondrial, microsomal and soluble fractions of rat liver. They reported that growth hormone did not alter the capacity of the liver of intact rats to incorporate cystine into protein, but that the incorporation into nuclear and mitochondrial fractions had increased as a result of the treatment with growth hormone.

The distribution of radioactive protein in these experiments (Friedberg & Greenberg, 1947; Lee & Williams, 1952) was examined several hours after administration of the labelled amino acid. Incorporation of amino acids into protein occurs very rapidly (Littlefield *et al.* 1955; Loftfield & Eigner, 1958) and the specific activity of the protein of those structures in the cell which are responsible for the assembly of amino acids into proteins will rise rapidly and fall again (if the intracellular amino acid specific activity drops) as the finished polypeptides are transferred from the template to other parts of the cell, or are secreted from the cell. The specific activities of proteins in other parts of the cell will also vary rapidly as a consequence of this shift of labelled material. The experiments reported in this paper were carried out with short time intervals between the administration of the radioactivity and the examination of the distribution of labelled protein in the cell. This difference in experimental design may help to explain the differences in the findings of Friedberg & Greenberg (1947) and Lee & Williams (1952), and those reported in this paper.

Balis, Samarth, Hamilton & Petermann (1958) found that treatment of intact male rats with growth hormone resulted in a more rapid incorporation of injected radioactive glycine into the pro-

teins of the microsomal particles of the liver. No rise in the extent of incorporation into these particles was noted after growth hormone treatment but this is not unexpected since the first time interval used was 15 min. after the injection of the radioactive amino acid, by which time much of any difference in specific activity between normal and treated rats would be expected to have disappeared (see Fig. 1). Balis *et al.* (1958) also reported that the specific activity of the proteins of the microsomal lipoprotein, combined with the protein of the soluble fraction of the cell, reached a maximum earlier than in untreated rats.

Ulrich, Tarver & Li (1954) found that hypophysectomy depressed the rate of turnover of serum albumin and that treatment of hypophysectomized rats with growth hormone restored the turnover rate almost to the normal level.

According to a recent personal communication, J. L. Kostyo and E. Knobil have observed diminished incorporation of injected radioactive leucine into tissue proteins after hypophysectomy. They found that administration of growth hormone to hypophysectomized rats increased amino acid incorporation only if the animals were fasted before injection of the radioactive amino acids. The rats used in most of the experiments described in this paper were effectively fasting for some hours before death (see Methods section), but a stimulating effect of growth hormone on amino acid uptake by tissues has also been noted in similar experiments carried out with fed rats. Fasting for 18 hr. did not alter the extent of incorporation of amino acids into protein in a system *in vitro* prepared from rat livers of normal or of hypophysectomized rats (Korner, 1959).

Location of the point of action of growth hormone. Some of the steps in the sequence of reactions which result in protein biosynthesis in the microsomal particles are known (see Hoagland *et al.* 1956; Hoagland *et al.* 1957; Hoagland, Stephenson, Scott, Hecht & Zamecnik, 1958; Littlefield *et al.* 1955). The control which is exerted by growth hormone, and by the other pituitary hormones, over the rate of biosynthesis of proteins could be exercised at one or more of these steps.

Size and composition of the free intracellular amino acid pool. The size of the amino acid pool inside the liver cell and the proportion of the amino acids of the pool which have arisen by entry into the cell, by controlled breakdown of tissue protein (Simpson, 1953; Korner & Tarver, 1957) or by synthesis of amino acids from carbohydrates, may well be under hormonal control. There is good evidence that growth hormone administered to the rat causes a fall in blood amino acid content (Griffith, Luck, Kulakoff & Mills, 1954; Li, Geschwind & Evans, 1949), and Russell (1955) has given some

evidence that the size of the amino acid pool in rat liver is decreased by treatment with growth hormone. Schaffer & Lee (1935) found that anterior pituitary-gland extracts decreased the free amino acid content of many tissues of the rat, including the liver. Li *et al.* (1949) found that hypophysectomy resulted in a fall in blood amino acid level but that this low level was not depressed much further by treatment of hypophysectomized rats with growth hormone. Hypophysectomy also resulted in a decrease in the size of the free amino acid pool of rat liver (Schaffer & Lee, 1935).

The manner in which growth hormone causes alterations in the size of the amino acid pool in rat liver is not clear. No evidence is available on the effect of growth hormone on the rate of entry of amino acids into liver cells from the blood. Nor, indeed, is there any evidence that a permeability barrier to amino acids exists in liver cells. The rate of gluconeogenesis is controlled by adrenal steroids, but not by growth hormone, and the evidence that growth hormone controls protein catabolism is not very strong (see Ketterer, Randle & Young, 1957). It is probable that the observed fall in pool size and in blood amino acid content of rats treated with growth hormone is a secondary phenomenon which occurs as a result of the increased rate of protein biosynthesis caused by the growth hormone.

The methods used in this paper to measure the intracellular specific activity of the free amino acids revealed no significant differences between normal, hypophysectomized and growth hormone-treated hypophysectomized rats. The variation between the results of individual rats in each group was large and differences in specific activity between the groups might be revealed by experiments carried out on a larger scale. The tendency for the specific activity of the free amino acids of the liver of hypophysectomized rats to remain at or near the initial value whereas the values for normal and growth hormone-treated hypophysectomized rats fell from the initial high level might be a result of the more rapid incorporation of amino acids into protein both in the liver and in peripheral tissues of the rats which have growth hormone available.

It appears that the differences in amino acid incorporation into protein found to exist between normal rats, hypophysectomized rats and hypophysectomized rats given growth hormone cannot be ascribed entirely, or even in large measure, to hormonally controlled changes in the intracellular amino acid concentration, and therefore in the specific activity of the amino acids available at the site of protein synthesis.

It is, however, possible that much of the amino acid available in the cell for protein biosynthesis is not free but is bound to activating enzymes,

soluble RNA or other cell components. If this is so, effects of hormones on the size of the pool of amino acids effectively available for protein synthesis would not be revealed by measurements of the free amino acid content of the cell.

Ability of the soluble fraction to prepare amino acids for incorporation into protein. The diminished incorporation of amino acids into microsomal protein in a liver cell-free system which follows hypophysectomy was shown to be brought about by a change in the liver microsomes resulting from hypophysectomy (Korner, 1958, 1959). It was pointed out that hypophysectomy may also have decreased the ability of the soluble fraction of liver to prepare amino acids for incorporation into protein, either by a fall in the activity of the amino acid-activating enzymes, or by a change in the rate of formation of the amino acid-soluble RNA intermediate, but that such changes may not be limiting and so would not be detected in the system used *in vitro*. In the experiments described in this paper, the lower maximum specific activity of the liver microsomal-particle protein of hypophysectomized rats can be ascribed to some change in the particles, resulting from hypophysectomy, which diminishes their ability to incorporate amino acids into proteins. The slower rise to the maximum specific activity shown by these particles from hypophysectomized rats may also be caused by some change in the particle which makes it more difficult for the activated amino acids to enter the particle or to find their correct locus on the RNA template. On the other hand, it is possible to argue that the cause is a slower preparation of the amino acids for incorporation into protein. Hypophysectomy may have caused a fall in the activity of the amino acid-activating enzymes, in the ability to form an amino acid-soluble RNA complex, in the ability to transfer the amino acid from the complex to the microsomal particle or in the supply of adenosine triphosphate or guanosine triphosphate needed for these processes.

Formation of polypeptide chains in the microsomal particle. The experiments described in Figs. 1-3 of this paper show that hypophysectomy has resulted in a reduced ability of the microsomal particles of rat liver to assemble amino acids into polypeptide chains. Treatment of the hypophysectomized rat with growth hormone can, to some extent, restore towards normal this reduced ability to synthesize protein. The other pituitary-gland hormones are probably also involved in the control of protein biosynthesis, for treatment of hypophysectomized rats with growth hormone alone has not succeeded in completely reversing the effect of hypophysectomy. This finding confirms the conclusions drawn from experiments *in vitro* (Korner, 1958, 1959).

Transfer of the synthesized peptide from the template to the lipoprotein of the endoplasmic reticulum. Twenty minutes after an injection of radioactive amino acids, a lower proportion of the total counts of the microsomal protein occurs in the lipoprotein portion of the endoplasmic reticulum in rats which have been hypophysectomized than is the case in normal rats, or in hypophysectomized rats treated with growth hormone (Table 4). This difference may be caused by a diminished ability of the synthesized polypeptides to peel off the RNA template of microsomal particles of livers of hypophysectomized rats. However, the effect is not manifest at times earlier than 20 min. after the injection of the amino acids and it is possible that the difference is not caused by any difference in the ability of the peptides to come off the template but is merely an effect of the maintenance of the specific activity of the intracellular amino acids at a high level for a longer time in the hypophysectomized rats than in the normal rat, which leads to the slower decline in the specific activity of the microsomal-particle protein and thus to a lower percentage of the counts in the deoxycholate-soluble protein of the livers of hypophysectomized rats.

Littlefield *et al.* (1955) showed that when high doses of radioactive amino acids were injected into rats, the specific activity of the microsomal-particle protein remained at a high level and did not fall off as was the case in experiments in which small 'pulse' doses of radioactivity were administered to rats. The slight tendency for the microsomal-particle protein from hypophysectomized rat liver to show this levelling off (Figs. 1 and 2) may be partly a result of the slower fall in the specific activity of intracellular free amino acids (Table 2), thus giving an apparently 'high dose' type of experiment.

SUMMARY

1. The incorporation *in vivo* of injected radioactive amino acids into the total protein of rat liver and into the proteins of the nuclei, mitochondria, microsomes and soluble fraction is diminished by hypophysectomy. Treatment of hypophysectomized rats with ox growth hormone restored, in part, the diminished incorporation of amino acids into the total proteins and into the proteins of the subcellular fractions of rat liver, towards the normal values. These changes in the amount of amino acid incorporation into proteins could not be ascribed to hormonally controlled changes in the specific activity of the intracellular amino acid pool.

2. Separation of the microsomes into microsomal particles and into material soluble in deoxycholate showed that there was a diminished

ability of the microsomal particles of liver from hypophysectomized rats to incorporate amino acids into protein. The maximum specific activity of the protein of these particles was depressed as a result of hypophysectomy and, in addition, this maximum value was attained at a longer time interval after injection of the radioactive amino acids than was the case with normal rats. Treatment of the hypophysectomized rats with growth hormone reversed both these trends to some extent.

3. The possible effects of hypophysectomy of the rat and of treatment of the hypophysectomized rat with growth hormone on different stages of protein synthesis are discussed.

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The Effect of the Administration of Insulin to the Hypophysectomized Rat on the Incorporation of Amino Acids into Liver Proteins *in vivo* and in a Cell-Free System

By A. KORNER

Department of Biochemistry, University of Cambridge

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It is generally accepted that insulin is a protein anabolic hormone. The rate of protein biosynthesis, measured in several ways, is subnormal in tissues of diabetic animals and can be restored towards the normal by treatment of the animals with exogenous insulin (see reviews by Lukens, 1954; Lukens & McCann, 1955 and Krah1, 1956). Some workers consider that insulin can exert its anabolic

action in the rat in the absence of growth hormone (Salter & Best, 1953; Lawrence, Salter & Best, 1954; Best, 1955; Salter, Davidson & Best, 1957; Griffiths, 1956; Lukens, 1958), but Wagner & Scow (1957) believe that exogenous insulin acts as a growth stimulant in hypophysectomized rats only by causing an increase in their food intake.