Effect of Hypophysectomy on the Rates of Protein Synthesis and Degradation in Rat Liver

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The effect of hypophysectomy on the protein metabolism of the liver *in vivo* was studied. Fractional rates of protein synthesis and degradation were determined in the livers of normal and hypophysectomized rats. Synthesis was measured after the injection of massive amounts of radioactive leucine. Degradation was estimated either as the balance between synthesis and accumulation of stable liver proteins or from the disappearance of radioactivity from the proteins previously labelled by the injection of $\text{NaH}^{14}\text{CO}_3$. The results indicate that: (1) hypophysectomy diminishes the capacity of the liver to synthesize proteins *in vivo*, mainly of those that are exported as plasma proteins; (2) livers of both normal and hypophysectomized rats show identical protein-degradation rates, whereas plasma proteins are degraded slowly after hypophysectomy.

It has been reported that hypophysectomy produces alterations in protein metabolism that lead to growth failure (Simpson *et al.*, 1949).

The liver, as well as other tissues from hypophysectomized animals, shows diminished ability to incorporate trace amounts of radioactive amino acids into proteins *in vivo* (Simpson *et al.*, 1949; Korner, 1960). The same results have been obtained for perfused liver (Jefferson & Korner, 1967), liver slices (Clemens & Korner, 1970; Liberti *et al.*, 1972) and cell-free systems (Korner, 1964). In spite of these reports, only two attempts have been made to estimate the liver protein-synthesis rates after hypophysectomy. Results obtained *in vivo* by Millward *et al.* (1975) show nearly normal rates, in contrast with the data of Feldhoff *et al.* (1977) on perfused liver showing that synthesis rates are decreased after hypophysectomy. The discrepancies in these studies could be due to the use (in experiments *in vivo*) of an isotopic technique that is not reliable for the measurement of protein synthesis in that the specific radioactivity of the amino acid precursor within the whole cell was determined instead of the specific radioactivity of the amino acid bound to tRNA, which is the real precursor pool (Khairallah & Mortimore, 1976).

The synthesis by the liver of albumin and several other plasma proteins has also been shown to be decreased after hypophysectomy by experiments *in vivo* (Ulrich *et al.*, 1954; Kernoff *et al.*, 1971) and in the perfused liver (Griffin & Miller, 1974; Feldhoff *et al.*, 1977). This decrease in the synthesis of plasma proteins is followed by a decrease in their catabolic rates (Ulrich *et al.*, 1954; Kernoff *et al.*, 1971).

With regard to liver protein-catabolic rates, Feld-

hoff *et al.* (1977) reported a decrease after hypophysectomy. This conclusion was reached from measurements of the difference between total protein synthesis and secreted-protein synthesis in perfused livers. The net increase in the protein in livers from normal control animals was not taken into account and it is possible that the catabolic rates were overestimated.

Since most of the studies described were performed *in vitro* and only investigated partial aspects of liver protein metabolism after hypophysectomy, it is possible that they do not give a quantitative account of the totality of the events that occur *in vivo*. The availability of reliable methods for the measurement of protein synthesis *in vivo* (Scornik *et al.*, 1978), and breakdown (Swick & Ip, 1974), makes a re-examination of the problem feasible.

In the present paper a detailed study is reported of the actual rates *in vivo* of liver protein synthesis and degradation in hypophysectomized rats.

Experimental

Diet

Rat chow (pellets) containing 23% (w/w) protein was obtained from Cargill S.A.C.I., Buenos Aires, Argentina.

Chemicals

L-[U- ^{14}C]Leucine (325 $\mu\text{Ci}/\mu\text{mol}$) and $\text{NaH}^{14}\text{CO}_3$ (46 $\mu\text{Ci}/\mu\text{mol}$) were from New England Nuclear Corp., Boston, MA, U.S.A. Non-radioactive L-leucine was purchased from Sigma Chemical Co.,

St. Louis, MO, U.S.A. Evans Blue was from J. T. Baker Chemical Co., Phillipsburg, NJ, U.S.A. Pactamycin was a gift from the Drug Research and Development Laboratory, Chemotherapy Branch, National Cancer Institute, Bethesda, MD, U.S.A. All other reagents were of the highest purity available.

Animals

Male Long-Evans rats from a strain bred in this laboratory were used. Where indicated, female animals of the same characteristics were used. They were housed, six per cage, in a 25°C room illuminated from 07:00 to 19:00h, and fed with rat chow and water *ad libitum*. When hypophysectomized, the rats were operated on at 45 days of age (100–120 g body wt.) and used 4 weeks later, unless indicated otherwise. Only the animals that maintained their initial weight ($\pm 10\%$) were used. Normal animals, matched in weight with hypophysectomized ones, were used as controls.

Analytical procedures

The collection of the livers, the determination of protein, RNA, rRNA and DNA, and the measurement of the radioactivity in liver proteins were as described by Scornik (1974a). Total plasma protein radioactivity was determined as described by Scornik & Botbol (1976). Plasma volume was determined by the Evans' Blue method (Furth & Sobel, 1946). The size distribution of liver polyribosomes from post-mitochondrial supernatants was analysed as described by Scornik (1973). Each gradient with 0.3 mg of RNA was run at 3°C for 3 h at 24000 rev./min in an SW 25 Spinco rotor (28 ml); 0.5 ml fractions were collected from the bottom of the tubes and their A_{260} and A_{320} values were determined. From the A_{260} value was subtracted the contribution of ferritin ($1.59 \times A_{320}$), and the resulting value was plotted as a function of the effluent volume. The proportion of leucine in liver proteins was determined in samples containing 2 mg of protein. The samples were homogenized and precipitated with 10% (w/v) trichloroacetic acid and submitted to the same treatments as those used for radioactivity measurements. After diethyl ether extraction, the pellets were allowed to dry at room temperature (20°C) and then hydrolysed for 24 h at 110°C in vacuum with 1 ml of 6M-HCl. A portion of the resulting material (0.05 mg) was analysed in a Technicon TSM-2 amino acid analyser.

Injected solutions

L-[U- ^{14}C]Leucine was injected in either trace or massive amounts. In the first case, the radioactive amino acid was diluted to 1 $\mu\text{Ci/ml}$ in 0.9% (w/v) NaCl, and adjusted to pH 7.4. In the second case, enough radioactive leucine was added to a 0.1M solution of non-radioactive leucine in 0.9% NaCl, pH 7.4, to give a final specific radioactivity of 0.01 $\mu\text{Ci/}$

μmol . $\text{NaH}^{14}\text{CO}_3$ was diluted in previously boiled 0.9% NaCl to give a final radioactivity of 50 $\mu\text{Ci/ml}$. Pactamycin, an inhibitor of the initiation of protein synthesis, was dissolved at a concentration of 2 mg/ml, at pH 4.5–5.0, as described by Scornik (1974b).

Results and Discussion

Composition of livers

The composition of the liver of hypophysectomized and normal rats is shown in Table 1. Clearly, hypophysectomy markedly decreases the RNA and, to a lesser extent, the protein and DNA content of the liver. As shown by the protein/DNA ratio, it seems that cell size does not change. These results agree with those reported by Jasper & Brasel (1973). In a separate experiment (results not shown), the livers of 15 hypophysectomized and 15 normal female rats were analysed. The values obtained were essentially the same as those in Table 1. No variation in the recorded values (Table 1) was observed when another group of 20 hypophysectomized rats was tested for liver composition 14 weeks after surgery (results not shown). The situation here is quite different from that of protein-depleted mice, whose liver protein content decreases rapidly to 45% of normal after 10 days of protein deprivation (Conde & Scornik, 1976). It seems that some kind of 'adaptive response' operates after hypophysectomy, as pointed out by Kernoff *et al.* (1971).

It should be noted that normal control animals are still growing. In a control experiment (results not shown) their fractional rate of liver protein growth was determined as described by Scornik (1974a). It was 0.037 mg of protein/day per mg of protein (Table 3, line *f*).

Protein synthesis

The rate of protein synthesis in hypophysectomized and normal rats was measured *in vivo* after the injection of massive amounts of radioactive leucine (Scornik, 1974a). Fig. 1 shows a plot of the reciprocal of the amount of radioactivity incorporated against the reciprocal of the amount of [^{14}C]leucine injected.

Table 1. Protein, RNA and DNA content of the livers from hypophysectomized and normal rats

Values (mg/100 g body wt.) represent means $\pm$ S.E.M. for the numbers of animals indicated in parentheses. For further details see the Experimental section.

Measurement	Hypophysectomized (41)	Normal (47)
Protein	624.0 $\pm$ 8.0	781.0 $\pm$ 9.0
RNA	21.5 $\pm$ 0.3	34.2 $\pm$ 0.4
DNA	8.9 $\pm$ 0.1	10.8 $\pm$ 0.1
RNA/protein	0.0345	0.0438
Protein/DNA	70.0	72.3

Fig. 1. Incorporation of massive amounts of L-[U- ^{14} C]-leucine

Animals received intravenously 50–300 μmol of leucine/100g body wt. and were killed 5 min after the injection. The reciprocal of the liver protein radioactivity expressed as d.p.m./min per mg of RNA is plotted versus the reciprocal of the amount injected. ●, Hypophysectomized rats; ○, normal rats. Each point represents at least four animals, and s.e.m. values are shown by bars unless too small to be represented. For further details see the text.

The data fit straight lines. From the intercepts at the ordinate the maximum rate of incorporation was calculated to be 76.5 d.p.m./min per mg of RNA in hypophysectomized and 123.4 d.p.m./min per mg of RNA in normal rats. With these values the synthesis efficiency was calculated by the following equation:

$$\text{Synthesis efficiency} = \frac{V_{\max.}}{S \cdot F_{\text{Leu}}} \quad (1)$$

where $V_{\max.}$ is the maximum rate of incorporation into total protein expressed in d.p.m./day per mg of RNA, S is the specific radioactivity of the precursor (169.21×10^3 d.p.m./mg of leucine) and F_{Leu} is the fractional proportion of leucine in liver proteins (0.105 by wt.; Richmond *et al.*, 1963). Control experiments demonstrated that this value does not change with hypophysectomy. Samples of five livers for each experimental condition were analysed as described in the Experimental section. The values obtained ($\pm$ s.d.) were 0.101 ± 0.007 in hypophysectomized and 0.103 ± 0.004 in normal rats.

The synthesis efficiencies calculated by eqn. (1) were 6.15 mg of protein/day per mg of liver RNA in hypophysectomized rats and 10.0 mg of protein/day per mg of liver RNA in the normal control animals. In order to calculate the fractional rates of total liver protein synthesis (mg of protein/day per mg of liver protein), these values must be multiplied by the RNA/

protein ratios as shown in Table 3, line c. The results reported here agree with early reports in which hepatic protein synthesis *in vivo* seemed to be decreased after hypophysectomy (Simpson *et al.*, 1949; Korner, 1960). Furthermore, they are consistent with the synthesis efficiencies that can be calculated by combining the rates of protein synthesis in perfused liver reported by Feldhoff *et al.* (1977) with the total liver RNA content reported by Keller & Taylor (1976) (8.6 mg of protein/day per mg of liver RNA in hypophysectomized rats, and 13.2 mg of protein/day per mg of liver RNA in normal rats). It should be noted that the results of Feldhoff *et al.* (1977) were obtained by perfusing the livers with massive amounts of radioactive leucine, the same approach as used in the present work for measurements *in vivo*. The fractional rate of protein synthesis of 0.438 mg of protein/day per mg of protein (Table 3, line c) in the livers of normal rats differs from the value of 0.73 mg of protein/day per mg of protein reported by Henshaw *et al.* (1971). They injected large amounts of radioactive lysine and determined its intracellular specific radioactivity for the calculations (not extrapolated to infinity). An underestimation of the specific radioactivity of the intracellular lysine pool could give a high fractional rate of liver protein synthesis. The composition of the livers seems not to be responsible for the discrepancy discussed above, since the RNA/protein ratio as well as the lysine content (0.54 μmol of lysine/mg of protein; determined as for leucine content) of normal liver proteins reported in the present work agrees well with the values reported by Henshaw *et al.* (1971). On the other hand, they found that the fractional rates of liver protein synthesis depend on the growth rates of the animals. Their calculations are for animals growing at 6% per day. This could be the explanation for the difference pointed out above, since the average rate of growth of the normal rats studied in the present work is 3% per day. However, if we calculated the fractional rates of liver protein synthesis assuming their animals are growing at 3% per day, the resulting value is 0.26 mg of protein/day per mg of protein, which is lower than the value reported in the present work. The procedure applied extrapolates the free amino acid pool to infinity and has proved to be the simplest, both technically and conceptually (Scornik *et al.*, 1978). It obviates the determination of the specific radioactivity of the intracellular precursor pools, which, as pointed out by Clemens & Korner (1970), may not be the same as those of the functional pools within cells. It has been reported by Khairallah & Mortimore (1976) that these functional pools correspond to the aminoacyl-tRNA species. The higher synthesis efficiency in normal rats than in hypophysectomized rats could be explained by assuming that in normal rats a higher proportion of total RNA is rRNA. However, when groups of five animals in each experimental condition

were tested, the fraction of total RNA that was rRNA was found to be $60 \pm 2\%$ in hypophysectomized and $59 \pm 1\%$ in normal rats. These values are similar to those reported by Keller & Taylor (1976). It can be

Fig. 2. Sucrose-gradient analysis of ribosomes from livers of hypophysectomized (●) and normal (○) rats. Postmitochondrial supernatants of the pooled livers of five rats containing 0.3 mg of RNA were analysed in a linear sucrose gradient as described by Scornik (1973). Fractions were collected from the bottom of the tube.

concluded, then, that RNA is used with less efficiency by the livers of hypophysectomized rats.

The polyribosomal populations in the livers of normal and hypophysectomized rats are shown in Fig. 2. Quantification of the areas below the curves indicates that this fraction in hypophysectomized animals represents only 63–70% of the amount found in normal rats. The literature records conflicting results about the variation in polyribosome size after hypophysectomy: Korner (1964) found disaggregation, but Keller & Taylor (1976) did not. The results reported here support Korner's (1964) findings and are further sustained both by the diminished protein-synthesis efficiency found in the present work (60% of normal) and by that calculated from the work of Feldhoff *et al.* (1977) and Keller & Taylor (1976) (see above). This parameter should not have changed if the polyribosome size remained constant after hypophysectomy, since the elongation times seem to be unaltered under these circumstances (Feldhoff *et al.*, 1977).

Proportion of the newly synthesized proteins retained and secreted by the liver

It has been reported that in adult mice nearly half of the newly synthesized protein disappears from the liver within 180 min; about 30% is secreted as plasma proteins, whereas the remaining 20% represents rapidly turning-over liver proteins (Scornik & Botbol, 1976). In the present work the proportion of pulse-labelled proteins retained and secreted by the livers of normal and hypophysectomized rats was studied after the injection of a trace amount of L-[U- ^{14}C]leucine, followed 4 min later by pactamycin to interrupt the pulse. Pactamycin inhibits protein synthesis for at least 3 h. In a control experiment (results not shown) it was injected at zero time, followed 180 min later by a 5 min pulse of [^{14}C]leucine and no radioactivity was detected in total liver protein. Hence the experimental design permits the measurement of the protein radioactivity remaining in the

Table 2. *Proportion of the newly synthesized proteins retained and secreted by the liver*

At zero time, each rat received the intravenous injection of a trace amount of L-[U- ^{14}C]leucine (0.5 $\mu\text{Ci}/100\text{ g body wt.}$) followed by pactamycin intraperitoneally (1.6 mg/100 g body wt.) 4 min later. The animals were killed either 7 or 180 min after the injection of leucine and their total liver and plasma protein radioactivity was determined. Plasma volume was determined by the method of Furth & Sobel (1946). Results (d.p.m./ μCi injected) are means $\pm$ S.E.M. for the numbers of rats indicated in parentheses. *Difference from normal rat value significant at $P < 0.001$ (Student's *t* test).

Condition	$10^{-3} \times \text{Radioactivity (d.p.m./}\mu\text{Ci injected)}$			Retained (%)	Secreted (%)
	Liver		Plasma		
	7 min	180 min	180 min		
Hypophysectomized	64.16 ± 1.44 (10)	36.01 ± 1.01 (15)	12.90 ± 0.68 (5)	$56.12 \pm 1.33^*$	$19.10 \pm 0.60^*$
Normal	88.20 ± 2.02 (7)	32.80 ± 1.47 (12)	28.35 ± 1.11 (5)	37.18 ± 1.24	32.10 ± 1.30

liver 180 min after tracer injection when the secretion of the pulse-labelled plasma proteins is complete (Scornik & Botbol, 1976). Groups of rats were killed 7 min and 180 min after tracer injection and their total liver protein radioactivity was determined. Total plasma protein radioactivity was determined at 180 min. Results in Table 2 show that after 180 min the livers of hypophysectomized rats retain 56% as much radioactivity as is present 7 min after injection, whereas the normal controls retain only 37%. When these values were multiplied by the fractional rates of total liver protein synthesis (Table 3, line *e*), the fractional rates of synthesis of stable liver proteins were 0.120 mg of protein/day per mg of liver protein in hypophysectomized and 0.163 mg of protein/day per mg of liver protein in normal rats. The rate in hypophysectomized animals is equivalent to 74% of that in normal rats. Millward *et al.* (1975) observed that 7–10 days after hypophysectomy the synthesis of stable liver proteins was nearly normal compared with normal rats fed *ad libitum* and lower with respect to pair-fed normal controls. Hypophysectomized animals in the present work were studied 4 weeks after surgery. This situation could be different from that 7–10 days after surgery when the effects of pituitary insufficiency start to be demonstrable (Kernoff *et al.*, 1971). However, the observation that a decreased food intake (pair-fed normal rats) increases the ability of the livers to synthesize stable proteins differs from the results in mice reported by Conde & Scornik (1976, 1977). In the work of Millward *et al.* (1975) the rate of synthesis of stable liver proteins was measured by the constant-infusion method of Garlick *et al.* (1973), which considers the precursor specific radioactivity to be equal to the total free amino acid pool.

Table 2 shows that 19 and 32% of the total protein radioactivity incorporated into the livers at 7 min appear after 180 min in plasma proteins of hypophysectomized and normal rats respectively. The unaccounted for radioactivity (25% in hypophysectomized rats and 30% in normal rats) could be assumed to represent high-turnover proteins (Scornik & Botbol, 1976). Hence, although the livers of hypophysectomized animals only synthesize 49% of the

normal amount of protein (Table 3, line *c*), they retain proportionally more of the newly formed proteins. According to these results the synthesis of plasma proteins seems to be decreased in these animals. The values found here for the secreted proteins are close to those obtained by Feldhoff *et al.* (1977) on perfused liver, and are consistent with previous reports (Kernoff *et al.*, 1971; Griffin & Miller, 1974; Keller & Taylor, 1976). The last-mentioned authors reported a decrease in the amount of active albumin-synthesizing polyribosomes. Whether there is a decrease in the total albumin mRNA as well as other mRNA species coding for plasma proteins remains to be answered.

Protein degradation

Fractional rates of liver protein degradation were measured as the difference between the synthesis of stable liver proteins and the net protein gain (Scornik & Botbol, 1976; Conde & Scornik, 1976, 1977). Results are shown in Table 3, line *g*. The livers of both normal and hypophysectomized rats degrade proteins at the same rate.

Catabolic rates were also studied by the disappearance of radioactivity from the liver proteins labelled with NaH¹⁴CO₃. The advantages of this precursor in experiments of this kind have been discussed by Swick & Ip (1974). No differences were observed between the two experimental conditions (Fig. 3*a*). This result is consistent with that obtained by the balance procedure. On the other hand, Feldhoff *et al.* (1977) reported that liver protein-breakdown rate is decreased after hypophysectomy in perfused liver and isolated hepatocytes. An explanation for this discrepancy from the present results could be that Feldhoff *et al.* (1977) used for the calculations the difference between the total protein synthesized and the fraction exported. This value does not represent the fraction of newly synthesized proteins retained by the liver as stable components, since it includes a proportion of rapidly turning-over proteins (Scornik & Botbol, 1976) that probably represents a small amount of the total liver protein content. These proteins are synthesized more rapidly and would have a higher specific radioactivity after a short period of

Table 3. Estimation of protein-degradation rates as the difference between the synthesis of stable liver proteins and the net protein gain

Explanations are given in the text. Key for calculations: *c* = *a* × *b*; *e* = *c* × *d*; *g* = *e* − *f*.

Measurement	Units	Hypophysectomized	Normal
(a) RNA/protein ratio		0.0345	0.0438
(b) Synthesis	mg of protein/day per mg of RNA	6.2	10.0
(c) Total synthesis	mg of protein/day per mg of protein	0.214	0.438
(d) Fraction retained		0.561	0.372
(e) Synthesis of stable liver proteins	mg of protein/day per mg of protein	0.120	0.163
(f) Net protein gain	mg of protein/day per mg of protein	0.00	0.037
(g) Degradation	mg of protein/day per mg of protein	0.120	0.126

Fig. 3. Disappearance of radioactivity from liver and plasma proteins labelled with $\text{NaH}^{14}\text{CO}_3$

At zero time, rats were injected intraperitoneally with $25\mu\text{Ci}$ of $\text{NaH}^{14}\text{CO}_3$, and 5 min before they were killed they received an intravenous injection of Evans' Blue (2 mg). Groups of at least five animals were killed at the time intervals indicated on the abscissa and total liver and plasma protein radioactivity was determined. Results were expressed as percentage of the radioactivity 6 h after the injection. The S.E.M. is indicated by the bars, and the apparent rates of degradation (in parentheses) were calculated at each interval on the assumption that the processes follow first-order kinetics. (a) Disappearance of total liver protein radioactivity. ▲, Hypophysectomized rats; △, normal rats; 100% values were 39.1×10^3 d.p.m. in hypophysectomized and 62.0×10^3 in normal rats. (b) Disappearance of total plasma protein radioactivity. The average values of plasma volume were 4.7 ± 0.1 ml/100 g body wt. in normal rats and 3.6 ± 0.1 ml/100 g body wt. in hypophysectomized ones. ●, Hypophysectomized rats; ○, normal rats;

labelling (Arias *et al.*, 1969). In addition, the calculations made by Feldhoff *et al.* (1977) did not consider the net protein gain of the normal livers that are still growing. (The rats used were of 110–135 g body wt.) This value should be subtracted from the synthesis of stable liver proteins as shown in Table 3, line *g*.

The disappearance of radioactivity from plasma proteins labelled by the administration of $\text{NaH}^{14}\text{CO}_3$ is shown in Fig. 3(b). Hypophysectomy decreases the catabolic rates of these proteins. This finding is in agreement with previous reports (Ulrich *et al.*, 1954; Kernoff *et al.*, 1971). The latter authors postulated that the low plasma albumin breakdown rate in hypophysectomized rats is an adaptive response caused by the decline of its pool to 'some critical value' as a consequence of its lower rate of synthesis.

In conclusion, the non-growing livers of hypophysectomized rats synthesize protein more slowly and degrade it at the same rate as normal rat livers. Nevertheless, hypophysectomized animals preserve their liver mass, retaining more than 50% of the newly synthesized proteins. The lower rate of synthesis of plasma proteins that occurs after hypophysectomy is compensated by their diminished catabolic rate. The molecular mechanism by which these adaptive responses operate remains to be elucidated.

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100% values were 21.6×10^3 d.p.m. in hypophysectomized rats and 40.6×10^3 in normal ones.

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