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The Effect of Starvation on the Rate of Protein Synthesis in Rat Liver and Small Intestine

By MARGARET A. McNURLAN, ANDREW M. TOMKINS and PETER J. GARLICK
Department of Human Nutrition, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, U.K.

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1. A method is described that allows for measurement of protein synthesis in liver and intestine in the rat. By injecting a massive amount of [^{14}C]leucine (100μmol/100g body wt.) an attempt has been made to overcome problems of precursor specific radioactivity and problems arising from the breakdown of labelled protein that are encountered when tracer amounts of amino acids are used. 2. Starvation for 2 days resulted in a decline in the rate of total liver protein synthesis from 87%/day to 62%/day. 3. In jejunal mucosa the rate of protein synthesis was 136%/day. This declined to 105%/day after 2 days of starvation.

Both liver and intestine are periodically subject to a large influx of amino acids. Since amino acid supply has been suggested as a means of influencing protein synthesis (Munro, 1970), the present study was undertaken to see if removing the amino acid supply, i.e. starvation, might result in a decrease in the rate of protein synthesis in these tissues.

A decrease in the rate of liver protein synthesis in response to starvation has been inferred from changes in polyribosome profiles (Munro *et al.*, 1975), but direct measurement of this effect is much more difficult. Measurements based on incorporation of a labelled amino acid *in vivo*, which have taken account of the specific radioactivity of the precursor, have shown little or no effect either on the total rate of synthesis of hepatic plus secreted proteins or on the synthesis of hepatic proteins only. Peters & Peters (1972) measured total liver synthesis from incorporation of labelled leucine over a 16min time period. They found that 18h of starvation had no effect on the fractional rate of protein synthesis (that is the percentage of the protein pool synthesized per day). Constant intravenous infusion of a labelled amino acid over several hours (Waterlow & Stephen, 1968) ensures that the specific radioactivity in the precursor is unchanging, but in liver it measures only intracellular proteins and not those that are secreted. By this method Garlick *et al.* (1975) reported a 15% decrease in the fractional rate of synthesis of hepatic tissue protein after 2 days of starvation.

In the present study the effect of starvation on total hepatic synthesis was investigated by measuring the incorporation over 10min of a large amount of labelled amino acid, a method similar to that first described by Henshaw *et al.* (1971). The mean specific radioactivity of the precursor was derived from the change in specific radioactivity of the precursor with time. This derivation is much easier with a large dose of labelled amino acid than if a tracer dose of amino acid is given, when complex changes in specific radioactivity must be analysed (Peters & Peters, 1972). The short time of incorporation ensures that total liver synthesis is measured. By measuring protein synthesis in this way we found a 30% decrease in the fractional rate of protein synthesis after 2 days of starvation.

The intestine responds to starvation with a marked loss of mucosal protein (McManus & Isselbacher, 1970) and a decrease in cell proliferation (Diller & Blauch, 1946; Hooper & Blair, 1958; Brown *et al.*, 1963; Altman, 1972; Hopper *et al.*, 1972; Löhrs *et al.*, 1974). Problems of measuring protein synthesis in the gut are numerous (Alpers & Kinzie, 1973). As with the liver, trace-labelling techniques must identify the specific radioactivity of the precursor. Possible precursors include not only plasma and tissue free amino acids but also intraluminal amino acids. Experimental evidence exists for all three possibilities. Studies *in vitro* by Fern *et al.* (1971) have suggested that the plasma is the source of amino acids for gut protein synthesis, whereas experiments *in vivo* (Alpers & Thier, 1972; Fern & Garlick, 1974) have suggested an intracellular source. Hirschfield & Kern (1969) have demonstrated that the incorporation of a labelled amino acid was different if the tracer was given systemically from that if it was given intraluminally. Alpers (1972) has resolved this apparent conflict by showing that plasma supplies the amino acids to the crypt region of the intestine and that luminal amino acids supply the villus region.

In addition to uncertainty about precursor, measurements made by constant infusion of labelled amino acid over several hours are also subject to error that arises because of loss of labelled protein by turnover during the period of infusion (James *et al.*, 1971).

Correcting for this loss is quite difficult because of the complex nature of protein breakdown in the gut. In addition to intracellular turnover, whole cells are exfoliated, so loss of label from protein is not random. However, by using a massive amount of labelled amino acid and measuring incorporation over only 10min, these difficulties can be avoided and the rate of protein synthesis in the intestine can be measured more reliably than with the constant infusion. A preliminary report of this method has been made previously (McNurlan *et al.*, 1978). The rate of protein synthesis in jejunal mucosa measured with the large dose of labelled leucine declined by 30% after 2 days of starvation.

Methods

All experiments were carried out on male Wistar albino rats purchased from Charles River (Margate, Kent, U.K.) at 50g body wt. and maintained on a cubed diet containing 23% (w/w) crude protein (Oxoid Ltd., Basingstoke, Hants, U.K.) until they reached 100g. In the starvation experiment the control group was killed at 100g body wt., whereas the starved group was kept for a further 48h, without food, before being killed. Radioisotope (from The Radiochemical Centre, Amersham, Bucks., U.K.) was administered via a lateral tail vein to unanaesthetized animals, which were wrapped in a cloth. Tracer doses (2μCi/rat) of L-[U-^{14}C]glycine (10mCi/mmol) and L-[U-^{14}C]lysine (10mCi/mmol) were given in 1ml of 0.9% NaCl. For the large dose, L-[1-^{14}C]leucine (50mCi/mmol) was combined with unlabelled leucine to give 10μCi and 100μmol of leucine/ml of water. Animals were killed by decapitation and blood was collected. Liver and jejunal mucosa were quickly removed, weighed and homogenized with 5vol. of either 10% (w/v) trichloroacetic acid (for measurements of leucine specific radioactivity) or 2% (w/v) $HClO_4$ (for tissue-composition determinations). Jejunal mucosa was obtained by removing the proximal 50% of the small intestine, which was flushed with cold 0.9% NaCl, everted and scraped with a microscope slide. Measurements of length were made on intestine that was stretched by the addition of a 3.5g weight.

Tissue composition was determined by the procedure of Lowry *et al.* (1951) for protein, a modified Schmidt–Thannhauser method for RNA as described by Munro & Fleck (1969) and the diphenylamine method as modified by Giles & Myers (1965) for DNA.

Trichloroacetic acid was removed from the supernatant of plasma and tissue homogenate by diethyl ether extraction and the specific radioactivity of leucine was determined on an automated amino acid analyser with a pump that divided the column effluent (Locarte, London W.12, U.K.). The specific radioactivity of protein-bound leucine was determined similarly after extensive washing of the precipitate with unlabelled leucine and trichloroacetic acid followed by hydrolysis in 6M-HCl at 110°C for 24h. All measurements of radioactivity were made on a Packard Tri-Carb liquid-scintillation counter in a Triton X-100/xylene-based scintillator (Fricke, 1975).

Calculations

Calculation of the rate of synthesis, k_s, in percentage per day was from the equation:

$$k_s = \frac{S_B}{\bar{S}_A t} \times 100$$

where S_B is the specific radioactivity of leucine in protein, $\bar{S}_A$ is the mean specific radioactivity of leucine in the precursor pool and t is the time expressed in days.

The method of Henshaw *et al.* (1971) assumes that the specific radioactivity of the precursor is constant over 20min. In experiments with [^{14}C]leucine reported here, this was found not to be the case, but the decline in specific radioactivity was linear with time (see the Results section). Because of this linear change, the mean specific radioactivity over 10min is simply the arithmetic average of S_A at zero time and 10min. S_A at zero time was determined by measuring the free-leucine specific radioactivity in groups of animals killed at 2 and 10min after injection and extrapolating the decline back to zero time. Independent determinations were made for control and starved groups. Determining S_A at zero time in this way assumes that the rate of decline between zero and 2min is the same as the rate measured between 2 and 10min. This assumption seems reasonable, since the rate of synthesis calculated over the time interval 0–2min was the same as the rate calculated over 2–10min. Calculating in this way has the advantage that a separate rate is determined for each animal in the 10-min group.

Alternatively, k_s could be calculated from the two groups of animals by measuring the change in specific radioactivity in the protein (S_B) and the precursor (S_A) between 2 and 10min. This calculation gives the same result as the calculation using the extrapolated value for S_A, but assessment of error is much more difficult, since k_s is determined from the means of two variables (S_B and S_A) at two time points (2 and 10min). Determining the specific radioactivity of leucine in protein after 2min of incorporation is also difficult to do accurately without adding a large and costly amount of radioisotope.

The specific radioactivity of the precursor (S_A) was taken to be the specific radioactivity of free leucine from the tissue homogenate (see the Discussion section), although for comparison k_s has also been calculated by using the specific radioactivity of leucine in plasma as the precursor.

Results

Precursor specific radioactivity

The time course for the change in specific radioactivity of leucine in plasma, acid-soluble supernatant from the tissue homogenate and protein is shown for liver (Fig. 1*a*) and jejunal mucosa (Fig. 1*b*) in rats given a large dose of leucine (100μmol/100g body wt.). Although the dose was not sufficiently large to flood all possible precursor pools to the same and constant specific radioactivity, the linear fall allows calculation of the mean specific radioactivity over the first 10min from measurements made at only two time points. Because the difference between the specific radioactivity of free leucine in plasma and tissue homogenate is much smaller than with the constant infusion, calculation of the synthesis rate (Table 1) from either potential precursor gives results that are much closer than the corresponding results obtained by Garlick (1972) who used constant infusion of a tracer dose of [^{14}C]tyrosine for 6h.

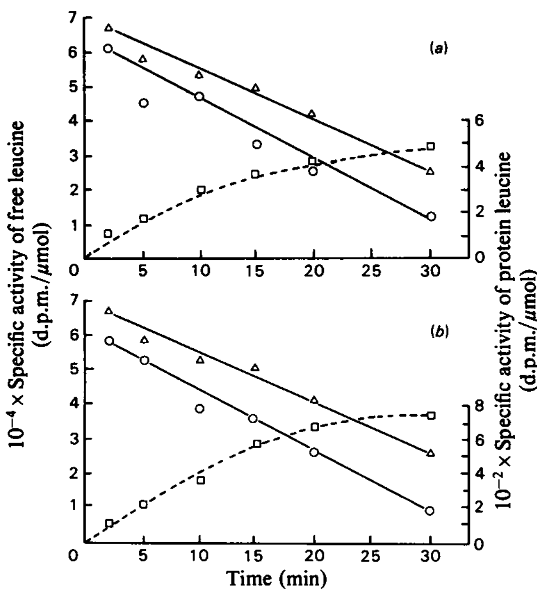

Fig. 1. *Time course for specific radioactivity of free leucine in plasma* (△) *and tissue homogenate* (○) *and of protein-bound leucine* (□) *in liver* (*a*) *and jejunal mucosa* (*b*)

Rats were injected with 100μmol of L-[1-^{14}C]leucine/100g. The specific radioactivity of leucine was determined as described in the Methods section for five animals at each time point.

Protein synthesis in the presence of a massive amount of leucine

Although the large dose of leucine eliminated some of the precursor problems encountered with tracer amounts of labelled amino acids, it was important to show that it did not affect the rate of protein synthesis. Three experiments were undertaken to look at the incorporation of a tracer dose of a different labelled amino acid in the presence and absence of the large amount of leucine. The results are given in Table 2. In Expt. 1 a tracer dose of [^{14}C]lysine was injected at the same time as the large amount of unlabelled leucine and incorporation at the end of 10min was measured. In the jejunal mucosa, incorporation was decreased by about 10% and in liver incorporation was decreased by about 20%. In Expt. 2 the tracer dose of [^{14}C]lysine was injected 2min after the large dose of leucine to minimize any decrease in incorporation that might be the result of suppression of transport of [^{14}C]lysine into the cell by the large amount of leucine. When the tracer dose of [^{14}C]-lysine was given after the large amount of leucine, incorporation of lysine was enhanced in both liver and jejunal mucosa by about 25%. Expt. 3 was similar to Expt. 1 in that the tracer amino acid, [^{14}C]glycine in this case, was given with the large dose of leucine. The difference in incorporation in the presence and absence of leucine in Expt. 3 was much smaller, being 10% in liver and only 5% in jejunum.

Effect of starvation

Starvation for 2 days resulted in a marked loss of body weight (Table 3). Although the liver weight was

Table 1. *Fractional rates of protein synthesis* (*in %/day*, ±S.E.M.) *in liver and jejunal mucosa*

Estimates of the fractional rates of protein synthesis by constant infusion of [^{14}C]tyrosine over 6h were taken from a previous study (Garlick, 1972). For estimates of the rate of protein synthesis by the large-dose method, rats were injected with 100μmol of [^{14}C]leucine/100g body wt. and the specific radioactivity of free leucine in plasma and tissue homogenate and the protein-bound leucine was determined in groups of five animals killed at 2 and 10min as described in the Methods section. Synthesis rates are shown with either the plasma or the tissue free leucine as the precursor.

	Constant infusion		Large dose	
Precursor ...	Plasma tyrosine	Tissue tyrosine	Plasma leucine	Tissue leucine
Liver	21 ± 1	59 ± 5	77 ± 6	87 ± 7
Jejunal mucosa	50 ± 17	218 ± 92	104 ± 13	136 ± 15

dramatically reduced, the protein concentration in the tissue was actually increased, so that the protein content of the organ per 100g initial body wt. was decreased by about 20 %. The loss of RNA from the liver exceeded the loss of protein by 15 % and the change in DNA content was negligible.

The length of the intestine was not altered by starvation, so comparisons have been made per cm of length. There was a 30 % reduction in weight and a parallel change in protein content (mg/cm). As with the liver, the decrease in RNA exceeded the decrease in protein, but in contrast to the liver, the DNA content of the jejunal mucosa was decreased.

The effect of starvation was to reduce the fractional rate of protein synthesis in both liver and jejunal mucosa (Table 4). This reduction in synthesis rate

Table 2. *Incorporation (d.p.m./mg of protein, ±S.E.M., $n = 5$) of tracer amino acids in the presence and absence of 100 µmol of unlabelled leucine*

Unlabelled leucine (100µmol/100g body wt.) was injected with a tracer dose (2µCi/rat) of [^{14}C]lysine (Expt. 1) or [^{14}C]glycine (Expt. 3) and incorporation into protein was measured at the end of 10min. In Expt. 2 the tracer dose of [^{14}C]lysine was injected 2min after unlabelled leucine (100µmol/100g rat) and incorporation was measured between 2 and 10min. None of the differences between the tracer alone and tracer plus leucine were found to be statistically significant by Student's t-test.

	Liver		Jejunal mucosa	
	Tracer alone	Tracer + leucine	Tracer alone	Tracer + leucine
Expt. 1 Leucine + [^{14}C]lysine	148 ± 12	123 ± 19	448 ± 27	401 ± 21
Expt. 2 Leucine, then [^{14}C]lysine	105 ± 9	130 ± 8	141 ± 8	182 ± 26
Expt. 3 Leucine + [^{14}C]glycine	456 ± 27	403 ± 40	553 + 44	524 ± 53

Table 3. *Changes in body weight and tissue composition in response to 2 days of starvation*

Tissue composition was measured as described in the Methods section in ten control animals and ten animals that had been starved for 48h. Statistical analysis of the difference between the control and the 2-day-starved group was by Student's t test; NS, not significant ($P > 0.05$).

	Control	Starved	
Body wt. (g, ±S.E.M.)	102 ± 4	74 ± 3	$P < 0.001$
Liver (mg/100g initial body wt., ±S.E.M.)			
Weight	4540 ± 77	2380 ± 110	$P < 0.001$
Protein	602 ± 34	487 ± 35	$0.02 < P < 0.05$
RNA	34.2 ± 1.9	22.2 ± 1.2	$P < 0.001$
DNA	7.54 ± 0.35	7.11 ± 0.21	NS
Jejunal mucosa (mg/cm, ±S.E.M.)			
Weight	30 ± 1	19 ± 1	$P < 0.001$
Protein	2.88 ± 0.14	2.04 ± 0.10	$P < 0.001$
RNA	0.26 ± 0.01	0.15 ± 0.01	$P < 0.001$
DNA	0.13 ± 0.01	0.11 ± 0.01	$0.02 < P < 0.05$

Table 4. *Effect of 2 days of starvation on the rate of protein synthesis in liver and jejunal mucosa*

The fractional rate of protein synthesis, k_s, in %/day (±S.E.M., $n = 5$) was measured in control and 2-day-starved animals after injection of 100µmol of [^{14}C]leucine/100g body wt. The difference in k_s between control and starved animals was assessed by Student's t-test; $0.01 < P < 0.02$ for both liver and jejunal mucosa. The absolute synthesis rate in mg/day for liver and mg/day per cm for jejunal mucosa is the fractional rate multiplied by the protein content per liver or per cm for jejunal mucosa. The difference between control and starved is $0.02 < P < 0.05$ for liver and $0.001 < P < 0.01$ for jejunal mucosa. Synthesis/RNA is the fractional synthesis rate divided by the RNA (mg per mg of protein) content of the tissue. For both liver and jejunum the difference between control and starved is not significant.

	Control			Starved		
	k_s (%/day)	Absolute synthesis rate (mg/day or mg/day per cm ±S.E.M.)	Synthesis/RNA (mg of protein/mg of RNA, ±S.E.M.)	k_s (%/day)	Absolute synthesis rate (mg/day or mg/day per cm, ±S.E.M.)	Synthesis/RNA (mg of protein/mg of RNA, ±S.E.M.)
Liver	87 ± 3	564 ± 51	15.5 ± 0.90	62 ± 7	342 ± 74	15.2 ± 1.5
Jejunal mucosa	136 ± 7	3.66 ± 0.26	14.1 ± 2.3	111 ± 6	2.30 ± 0.16	14.7 ± 1.6

could have been accomplished by the loss of protein-synthesizing machinery, i.e. RNA, or by a decrease in the amount of protein synthesized per mg of RNA (termed by Millward *et al.*, 1975, the 'activity of RNA'). Since there is little difference in the rate of synthesis of protein per mg of RNA between the control and the starved rats (Table 4), nearly all of the decrease in synthesis rate in both liver and jejunal mucosa can be accounted for by the decrease in the amount of RNA in the tissues.

Discussion

Rates of protein synthesis in liver and jejunal mucosa

An attempt has been made to measure the rate of protein synthesis in two tissues that turn over rapidly by a method that minimizes problems of precursor specific radioactivity and problems arising from the breakdown of labelled protein during the course of the experiment. That turnover was high is shown by the rapid decline in specific radioactivity of free leucine in both liver and jejunum, which occurred even though the free leucine pool was expanded to approximately 10 times the normal amount. The specific radioactivity was not constant, but the fact that the decline was linear with time makes measurements of the precursor much easier than with the complex changes in specific radioactivity produced by injecting a tracer amount of labelled amino acid (Peters & Peters, 1972). A choice of precursor is still involved in calculating the rate of synthesis from the specific radioactivity of free leucine in either the plasma or the tissue homogenate. However, the rates are not nearly as discrepant with the large-dose method as when a similar comparison is made for a trace-labelling method (Table 1). Support for choosing the tissue free leucine as the precursor is gained from the shape of the curve for leucine specific radioactivity in protein. Since this curve would be linear for a precursor of constant specific radioactivity, the fact that it is already flattening off at 30min suggests a precursor in which the specific radioactivity is rapidly declining. As Fig. 2 demonstrates the theoretical protein specific radioactivity curve derived from the leucine specific radioactivity in tissue homogenate resembles the actual protein curve quite closely.

This method assumes that the large amount of leucine does not alter the rate of protein synthesis. This assumption was tested by comparing the incorporation of tracer amounts of other amino acids in the presence and absence of the large amount of leucine (Table 2). Although in Expt. 1 and Expt. 2 there was an alteration in the incorporation of [^{14}C]lysine by the large amount of unlabelled leucine, it seems most unlikely that the decrease in incorporation in Expt. 1 and the enhancement of incorporation in Expt. 2 were both due to changes in the rate of protein

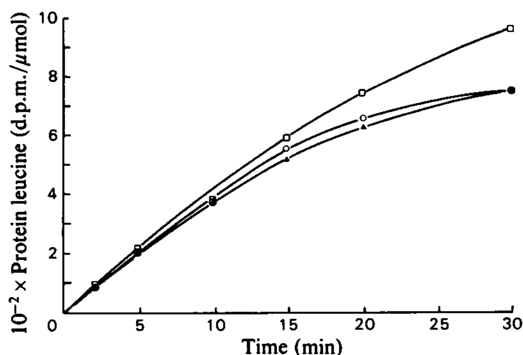

Fig. 2. *Comparison of observed specific radioactivity of leucine in protein of jejunal mucosa* (○) *with calculated specific radioactivity of leucine in protein with plasma* (□) *or tissue* (▲) *free amino acid as precursor*

The calculated specific radioactivity of leucine in protein was derived from the equation in the Calculations section by using either plasma or tissue free leucine as the precursor. The values of k_s used were those calculated from the 10min time point, i.e. 95%/day for plasma and 102%/day for tissue free amino acid. The precursor specific radioactivity was taken to be the mean of the measured specific radioactivity and the extrapolated (zero time) specific radioactivity. The values for observed specific radioactivity of leucine in protein were taken from Fig. 1(*b*).

synthesis. A more likely interpretation is that transport of [^{14}C]lysine has been altered by the presence of the leucine. Christensen (1969), in describing systems of transport for amino acids, discusses competition between amino acids such as leucine and lysine that would account for decreased uptake if the two amino acids were given together (Expt. 1). He also describes exchange reactions that would enhance the uptake of an amino acid given to cells preloaded with a different amino acid (Expt. 2). In Expt. 3, [^{14}C]glycine was chosen as the tracer amino acid, since one would not expect glycine to be transported by a system that also transports leucine (Christensen, 1969). The equal incorporation of [^{14}C]glycine with and without leucine seems to confirm the assumption that leucine was having no effect on the rate of protein synthesis.

The rate of protein synthesis in liver obtained with this method (87%/day) is in good agreement with the results of others. Henshaw *et al.* (1971) injected a large dose of [^{14}C]lysine and assumed that the precursor specific radioactivity was unchanged over the period of measurement. They obtained a value of 78%/day for total liver protein synthesis. Peters & Peters (1972) obtained values of 92%/day (measured at 10:00h) and 116%/day (measured at 22:00h) for total liver synthesis by using a tracer amount of [^{14}C]leucine and determining the change in precursor

specific radioactivity over 16 min. The constant infusion of a tracer amount of [^{14}C]tyrosine used by Garlick *et al.* (1975) measures only intracellular liver proteins and not those secreted, so the values obtained, 53–68 %/day, are lower than estimates of total liver synthesis.

Reliable measurements of the rate of protein synthesis in intestine have not previously been made. If one assumes the cell lifespan to be 41 h, as measured by Gleeson *et al.* (1972), then the fractional rate of protein synthesis must be at least 60 %/day just to replace cells that are lost. The measured rate of 136 %/day means that jejunal mucosa is synthesizing protein at a rate 50 % faster than that in liver. This high rate of protein synthesis also means that protein synthesis in the mucosa of the small intestine makes a significant contribution to whole-body synthesis, despite the fact that the small intestine is a rather small percentage of whole-body mass. Whole-body synthesis in rats weighing 100 g has been estimated to be 4.4 g of protein/day (Garlick *et al.*, 1975). The protein mass for the mucosa of the small intestine is about 440 mg. If this is turning over at 136 %/day, then the amount of protein being synthesized is about 600 mg or about 14 % of the total. By comparison liver has a protein mass of about 600 mg and a synthesis rate of 87 %/day, so the liver is synthesizing about 500 mg of protein, which is about 12 % of the total for the whole animal.

Effect of starvation on liver and jejunal mucosa

An animal deprived of food rapidly loses weight. The initial rapid weight loss is probably due to the loss of gut content, which is about 10 g in rats of this size. The response in individual tissues is quite variable. Liver and jejunal mucosa are decreased by 50 and 30 % respectively (Table 3) whereas muscle, heart, brain and kidney change very little (Garlick *et al.*, 1975).

In liver and jejunal mucosa, comparatively large changes in organ weights were accompanied by an overall loss in tissue protein and RNA. It is noteworthy that the loss of RNA exceeded the loss of protein in both these tissues. The amount of protein synthesized per mg of RNA (Table 4) was not changed very much by starvation, and in both tissues at least 90 % of the decrease in protein synthesis is accounted for by the decrease in RNA content. The combined effect of a decreased fractional rate of synthesis and a decreased amount of protein is shown by the fall in the absolute synthesis rate, that is, the total amount of protein being synthesized per day per liver or per cm of jejunum. After 2 days of starvation both organs were producing about 40 % less protein in total.

Total protein synthesis in liver includes both a component of synthesis of secreted proteins and a component for intracellular protein synthesis. Garlick *et al.* (1975) have shown that the fractional rate of synthesis of hepatic intracellular proteins was decreased by starvation. Peters & Peters (1972) measured the synthesis of albumin as a proportion of total liver synthesis. They observed that starvation produced a selective decrease in albumin synthesis. In contrast, in a study parallel to the present one, we found that during starvation albumin synthesis was not selectively decreased (Pain *et al.*, 1978). This would suggest that there were comparable reductions in the synthesis of intracellular and secreted proteins.

In the jejunal mucosa a reduction in the fractional rate of synthesis might be due to a decrease in the synthesis of secreted protein, a reduction in intracellular protein synthesis or a decrease in the rate of production of new cells. Although measurements of total synthesis cannot differentiate among the three possibilities, the magnitude of the change in protein synthesis is consistent with changes in cell turnover observed by others. To account for the observed decrease in fractional synthesis rate only in terms of a decrease in cell production would require that the proliferation of cells be decreased by half. Decreases of this magnitude have been reported by both Brown *et al.* (1963) and Löhrs *et al.* (1974) in the intestine of mice that had been starved for 2 days.

In conclusion there is a decrease in the rate of protein synthesis in both liver and jejunum after 2 days of starvation. Although only total synthesis is measured, assessment of the magnitude of the changes in the light of measurements made by others suggests that in liver there is a decrease in both secreted and intracellular protein. In intestinal mucosa it is possible that the decrease in synthesis is mainly due to a slowing of cell turnover, but more experimental evidence is needed before this can be concluded with certainty.

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