Effects of a Short-Term Fast on Albumin Synthesis Studied In Vivo, in the Perfused Liver, and on Amino Acid Incorporation by Hepatic Microsomes

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ABSTRACT Carbonate-^{14}C was used to label the hepatic intracellular arginine pool and direct measurement of albumin synthesis was made in six rabbits before and after an 18–36 hr fast. 18 perfusion studies were performed with livers derived from fed and fasted rabbits (18–24 hr). Microsomal amino acid–incorporating ability with leucine-^{3}H and phenylalanine-^{14}C was compared in 17 studies, using microsomes isolated from livers taken from fed and fasted rabbits and from isolated perfused livers whose donors were fed and fasted.

Albumin synthesis is rapidly inhibited by fasting. Albumin synthesis decreased 33% in vivo and 53% in the perfused liver. The microsomes from perfused livers taken from fed animals did not demonstrate a significantly reduced capacity to incorporate leucine-^{3}H or phenylalanine-^{14}C into protein. Microsomes derived from perfused and nonperfused livers whose donors were fasted incorporated 32–54% less tracer than microsomes obtained from fed donor rabbits. Microsomes separated from perfused livers removed from fed and fasted rabbits responded to polyuridylic acid stimulation and phenylalanine-^{14}C incorporation rose from 58 to 171%.

An 18–36 hr fast inhibits albumin production in vivo and in the perfused liver. The microsomal system is less active in the fasted state and perfusion per se does not inhibit the microsomal response.

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INTRODUCTION

Albumin synthesis has been found to be reduced in malnutrition (1–4) and these changes have been ascribed to a deficiency of available amino acids (3, 4). A decrease in polysomal size, as well as a more rapid turnover of RNA (5, 6), has been reported in the livers of fasted rats, and Marsh who has shown that perfused rat liver from a fasted rabbit made less protein ascribed this result to a deficient supply of glycogen (7). Thus, although fasting or deficient diets have been shown to inhibit albumin synthesis the mechanisms underlying this change in vivo and in vitro are not known and a correlation between in vivo and in vitro effects is not available. These questions were studied by observing the effects of an 18–36 hr fast in the whole animal, in the isolated perfused rabbit liver, and in subcellular systems derived from the in vivo and the perfused liver. Albumin synthesis in the intact liver and microsomal amino acid incorporation were measured, and the results indicate that a short-term fast inhibits protein synthesis in vivo and in vitro. Furthermore, microsomes from fasted animals are equally inhibited whether they are derived either from a perfused or an in vivo liver.

METHODS

(A) Whole animal studies

Six male rabbits of 4–5 kg were maintained on a 17% crude protein diet. They consumed 100–150 g/day and were studied twice, once when their food intake was permitted ad lib. and then 10 days later after an 18–36 hr fast. Albumin synthesis was determined by the injection of carbonate-^{14}C with its subsequent incorporation into the guanido carbon of arginine and, hence, into albumin. The specific activity of the carbon of urea was taken to represent that of the precursor guanido carbon of arginine as described previously (8–10).

Protocol. The rabbits' urinary bladders were catheterized and 3–5 μc of rabbit albumin-^{131}I was injected intravenously. After obtaining 1 ml of heparinized blood at 6 min, 500 μc of carbonate-^{14}C was injected intravenously, and 1-ml heparinized blood samples were obtained at 6–10 min and at 45 min intervals thereafter for 3.5–4.5 hr, starting 1.5 hr after the carbonate injection. At the end of this period a 10-ml sample was taken for the isolation of albumin. The plasma volume was determined from the space of distribution of rabbit albumin-^{131}I at 6 min and the net amount of albumin lost from the plasma during the 3.5–4.5 hr of the study determined from the difference between the 6 min and the 3.5–4.5 hr plasma albumin-^{131}I activity. The fractional rate of urea synthesis was determined from the rate of decrease of urea-^{14}C specific activity determined from a semi-logarithmic plot of the urea carbon specific activity of all five samples against time. In each of the 4 other rabbits, 30 μc of urea-^{14}C was injected 24 hr after the carbonate study and after 90 min, five heparinized blood samples were obtained at 45 min intervals, and urea synthesis was determined again.

Determination of urea carbon specific activity. The plasma samples were treated with tungstic acid and heated at 100°C to drive off CO_2-^{14}C. An aliquot for nitrogen analysis was incubated with urease according to the method of Conway and Byrne (11). Ammonia was released with 45% K_2CO_3, trapped in 2 N H_2SO_4, and assayed with Nessler reagent. A second aliquot was incubated with urease and CO_2 was released with H_3PO_4 and trapped in phenethylamine. The phenethylamine was dissolved in 15 ml of 0.7% butyl-PBD(-2-(4-*tert*-butylphenyl)-5-(4-biphenylyl)-1,3,4-oxadiazole) in toluene-methanol (1:1) and counted in an ambient temperature Nuclear-Chicago liquid scintillation counter.

Determination of albumin guanido carbon specific activity. Albumin was isolated from the last sample of blood by precipitation with 10% trichloroacetic acid (TCA). The precipitate was washed with 5% TCA and then extracted with 95% ethanol–1% TCA. Three parts of diethyl ether were added to the acid alcohol extract to precipitate albumin. The albumin was dissolved in dilute alkali and dialyzed overnight, in the cold, against distilled water.

The dialyzed contents were centrifuged and the supernatant was precipitated with TCA at a final concentration of 5%, reextracted with alcohol-TCA and again precipitated with 3 volumes of ether. The precipitate was dissolved in dilute alkali and dialyzed against cold distilled water. A sample was concentrated to 6% protein concentration and was qualitatively examined with cellulose acetate electrophoresis and agar gel immunoelectrophoresis to guard against the use of a preparation that contained obvious serum protein contaminants (12). The protein was hydrolyzed with 6 N HCl, neutralized, and passed through a resin column, according to the method of McFarlane, and treated consecutively with arginase and urease (10). To insure that the albumin isolated from the perfusate was not contaminated with hepatic protein, we examined immunochemically with sheep antiserum against rabbit albumin two protein fractions that had been isolated chemically, as above (13).

After the two samples were checked for purity by immunodiffusion and cellulose acetate electrophoresis, they were each divided into two portions. One portion was hydrolyzed and worked up enzymatically as described above, the second portion of each was precipitated with a slight excess of antiserum. The antibody-antigen complex was disassociated by the method of Peters (14), and the specific activity of the guanido carbon from the isolated albumin determined as above. One sample was from a perfusion of a liver from a fed animal, and the specific activity of the isolated albumin was high–6400 cpm/mg of guanido carbon (alcohol-TCA) and 6120 cpm/mg of guanido carbon (antibody separation). The other study was from a fasted preparation with low specific activities–1980 and 1910 cpm/mg of guanido carbon by the two methods, respectively.

Since 50–75 ml of antisera were required to precipitate enough albumin for each study, it was not routinely feasible to isolate albumin immunochemically.

In two other studies, the livers were subjected to surgical stress by impeding the outflow through the inferior vena cava. After 2.5 hr of perfusion when the livers were swollen and dark, albumin was isolated from the perfusate with alcohol-TCA as above. The guanido carbon specific activity was less than 5% of the activity found with a normal perfusion system. Thus, although it is possible that the albumin fraction isolated may indeed be contaminated with some hepatic or other protein, our studies indicate such contamination was minimal. Nitrogen and CO_2-^{14}C were determined as above.

The zero time urea-^{14}C specific activity was determined from the zero time intercept of the plasma urea-^{14}C curve and albumin guanido-^{14}C specific activity at zero time by correcting the 3.5–4.5 hr specific activity for that fraction albumin-(^{131}I) that had left the plasma.

Albumin synthesis was determined from the formula proposed by McFarlane (15).

(*a*) Fractional rate of albumin synthesis

$$= \frac{\text{specific activity albumin guanido-}^{14}\text{C at } T_0 \times \text{fractional rate of urea synthesis}}{\text{urea-}^{14}\text{C specific activity at } T_0}.$$

(*b*) Albumin synthesis (*gram*) = plasma albumin mass (*gram*) × A.

(B) Perfusion studies

18 perfusion studies were performed in livers removed from 10 fed rabbits and from eight fasted 18–24 hr before surgery. Under light ether anesthesia the stomach and esophagus were tied and severed. The bile duct was isolated and cannulated and bile was expressed to empty the gall bladder. The portal vein was cannulated and perfusion was started immediately at a rate of 15–30 ml/min from a 500 ml reservoir at a height of 40–50 cm. The inferior vena cava was cannulated from below the liver and ligated above the diaphragm. This portion of the surgery required about 15 min and the liver was without perfusion for only a few sec during the entire procedure. The liver was then removed and mounted on a platform in an humidified box maintained at 37°C. The perfusate in all studies consisted of two parts of fresh heparinized rabbit blood; one part oxygenated Krebs-Hensleit solution which contained 50–90 mg/100 ml of glucose; 3–4 μmoles of amino acids/ml, equivalent to fresh plasma, which included the essential amino acids; and 0.69 μmole of L-glutamine/ml and 0.24 μmole of L-arginine/ml were added (16). Perfusion was directed into the portal vein at a rate of 0.3–1.4 ml/g of liver per min. The perfusion volume of 110–130 ml was recirculated and oxygenation was carried out in a disk oxygenator that received the output from the inferior vena cava. Bile was collected from the cannulated biliary duct. After 15–30 min of perfusion, 100 μc of carbonate-^{14}C was injected directly into the inflow tube connected to the portal vein and the perfusion continued for 2.5 hr, at which time the total perfusate was collected. Albumin synthesis was calculated from the expression:

$$\text{albumin synthesis } (mg) = \frac{\text{total perfusate}}{\text{albumin } (mg)} \times \frac{\text{albumin guanido-}^{14}\text{C specific activity}}{\text{synthesized urea-}^{14}\text{C specific activity}}.$$

Synthesized urea was calculated from the difference between the initial and final urea content of the perfusate and the volume of distribution within the liver and red cells. These spaces (measured from the space of distribution of urea-^{14}C) were determined to be about 60%. The specific activity of this urea carbon was assumed to equal the mean specific activity of the precursor guanido carbon of arginine (8–10).

The concentration of protein in plasma or perfusate was determined with a biuret-reagent (17) and protein partition with a Kern microelectrophoresis unit (18). Albumin was isolated and purified as above.

Perfusion techniques. Due to the large size of the rabbit liver, 40–70 g, the volume required to match in vivo flows (19, 20) was often high with excessive hemolysis. Lower flow rates and volumes were easier to control with minimal hemolysis. Flows of 0.3–0.35 ml/g per min were chosen in 11 studies. Whereas the normal rabbit hepatic blood flow in vivo is between 0.7 and 1.4 ml/g per min (19, 20), most of this is derived from oxygen unsaturated blood. In the present system oxygenated blood was pumped through the portal system. These differences in flow made it necessary to characterize our system in terms of function other than protein production. The following criteria were used: (*a*) bile flow and urea production, (*b*) histologic examination, (*c*) O_2 consumption, and (*d*) lactate and pyruvate levels; and in six studies in livers from fasted rabbits, lactate utilization for glucose production was determined (21). (*a*) Bile flow remained constant in any one preparation and varied from 1.0–2.5 ml/hr, independent of the hepatic blood flow. Urea production averaged 91 ±8 mg/100 g during the 2.5 hr perfusion in the fed group and 100 ±14 mg in the livers from fasted rabbits, and no variation with flow rate was observed. (*b*) Histological examination failed to reveal any abnormality.[1] (*c*) O_2 consumption, measured in six representative preparations with perfusion rates of 0.3–1.2 ml/g per min averaged 1.2–1.4 ml/g per hr, a value close to that reported by Fischer (22). At the low flow rates (0.3–0.4 ml/g per min) the oxygen extraction was between 4.0–6.0 ml/100 ml. (*d*) Gluconeogenesis from lactate cannot be adequately measured in the livers isolated from fed rabbits due to release of glucose from glycogen; thus, the lactate studies were performed in rabbits fasted for 48 hr before the perfusion. After 60 min of perfusion at flow rates of 0.3 ml/g per min, 1000 μmoles of lactate were added to the perfusion system and lactate disappearance and glucose production determined while the low flow was maintained (23). At the start of the perfusion, lactate/pyruvate ratios averaged 40 ±2; after 1 hr the ratios decreased to 9 ±2 with lactate levels falling in each study. 1–1.5 hr after the addition of the excess lactate, 692 ±39 μmoles of lactate had been utilized and 377 ±42 μmoles of glucose had been formed.

In the perfused livers from fed donors a progressive rise in blood glucose, from 77 ±6 to 230 ±35 mg/100 ml, occurred in 12 studies over 2.5 hr. Studies of glycogen content of the liver did not reveal any significant loss. In the fasted state, glycogen levels were low and in four studies they ranged from 0.4 to 1.2 mg/g of liver. In two studies in livers from fed rabbits, glycogen levels ranged from 57 to 67 mg/g and were unchanged after perfusion, even though perfusate glucose levels rose more than 200 mg/100 ml. Two studies were performed to determine the effects of perfusion on hepatic glycogen reserve in livers from fasted animals at perfusion rates of 0.24 and 0.60 ml/g per min. After the 1st 60 min of perfusion 1000 μmoles of lactate were added and glucose production measured. In addition, a small lobe of the liver was removed before the perfusion and the glycogen content of the hepatic tissue determined (24). The perfusate sugar rose from 68 to 208 mg/100 ml and glycogen content at the end of the study was 5.1 mg/g of liver not depressed from the initial level. During the 1st 60 min of perfusion 390 μmoles of lactate were utilized and after the addition of 1000 μmoles of lactate 904 were utilized.

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In a second study, perfusate sugar levels rose from 72 to 292 mg/100 ml, whereas the glycogen level remained at 2.8 mg/g of liver before and after perfusion. The lactate/pyruvate ratio fell from 29/1 to 9/1.

(C) Microsome studies

Preparation of microsomes. After the perfusion, the liver was washed with 20-ml of ice cold saline and homogenized in 2.5 times its weight in a medium containing $MgCl_2$ (10 mM), KCl (25 mM), Tris buffer (35 mM, adjusted to pH of 7.8 with HCl), sucrose (0.15 M), and 2-mercaptoethanol (6 mM). The tissue suspension was centrifuged for 10 min at 15000 *g* and the supernatant was centrifuged for 1 hr at 105,000 *g* (No. 40 rotor) in a Spinco model L preparative centrifuge. The sedimented microsomes were gently resuspended in volume of homogenizing medium, such that the suspension contained approximately 10 mg of protein/ml. 11 studies were performed which compared leucine-^{3}H incorporation into protein by hepatic microsomes derived from perfused livers whose donors were fed and fasted with incorporation by hepatic microsomes from livers from intact fed and fasted rabbits. Six studies were performed with phenylalanine-^{14}C, using microsomes from perfused and nonperfused livers from fed and fasted animals. In order to determine if the alteration in synthesis seen in the fasted state could be augmented with exogenous messenger RNA (25), 400 μg of polyuridylic acid were used in each incubation tube to offset RNAase degradation by cell sap (26).

Method of incubation. Each incubation tube contained 0.4 ml of the microsomal suspension; 0.2 ml of the 105,000 *g* supernatant; leucine-^{3}H (18–25 μc/tube: SA, 5 c/μM) or ^{14}C-labeled amino acid (2.5 μc/tube: SA, 240 μc/μmole); 2 μmoles of ATP; 0.5 μmole of GTP; 20 μmoles of phosphoenolpyruvate; 56 μg of pyruvic kinase; 0.1 μmole each of 17 ^{12}C-labeled amino acids, excluding the labeled amino acid in a total volume of 1 ml. Incubations were at 37°C for 30 min. The reaction was stopped by the addition of 0.4 ml of 1 N NaOH. Control cell sap was chosen, because cell sap derived from the perfused livers decreased the ability of all microsomes to incorporate labeled amino acids into TCA precipitable material by 60–75%. The cell sap from the hepatic microsomal preparations obtained from fed and fasted donor rabbits did not alter the microsomal activity, and thus the experiments were grouped according to the microsomal preparation.

Assay procedure. 50-μl of the alkaline solution were pipetted onto 2.1-cm disks of 3MM Whatman filter paper. The disks were washed as described by Mans and Novelli (27). Each disk was suspended in 5-ml of a toluene-methanol (1:1) solution of butyl PBD (7 g/liter) and counted in a room temperature liquid scintillation counter.

Protein was determined by the Folin-Ciocalteu method of Lowry, Rosebrough, Farr, and Randall (28) with bovine serum albumin used as standard.

RNA was determined by the orcinol method of Mejbaum with yeast RNA used as standard (29).

RESULTS

The results of the in vivo studies in the six fed and fasted rabbits are shown in Table I.

(A) Whole animal studies. After the fast the rabbits' weight did not change, and there was no change in urea concentration, albumin levels, or plasma volume. Albumin synthesis decreased 33% from 290 ±26 to 193 ±17 mg/kg per day ($P < 0.01$).

(B) Perfusion studies. In three studies the albumin guanido carbon specific activity was determined after 75, 100, and 150 min of perfusion. At 75 min the specific activity was 1290 ±115 cpm/mg carbon. At 100 min the value increased 57% to 2025 ±40 and at 150 min the value was only 14% higher at 2310 ±160 cpm/mg carbon, indicating that albumin release had been essentially completed at this time.

The results of the perfusion studies in 18 rab-

TABLE I
Albumin Synthesis in Rabbits before and after an 18–36 hr Fast (Six Studies)

	Wt		Albumin		Albumin synthesis		Urea N		Plasma volume	
	Fed	Fast	Fed	Fast	Fed	Fast*	Fed	Fast	Fed	Fast
	kg		*g/100 ml*		*mg/kg per day*		*mg/100 ml*		*ml/kg*	
Mean	3.5	3.4	3.2	3.1	290	193	24.2	24.4	109	113
±SE‡	0.1	0.1	0.1	0.1	26	17	0.2	0.2	5	4
P value						< 0.01				

*No correction for residual activity in the isolated albumin was made. In three studies this persistence of the initial label amounted to 10–15% of the albumin guanido carbon specific activity observed after the second dose of carbonate-^{14}C.
‡SE, standard error of mean.

bit livers are shown in Table II. Albumin synthesis was depressed 53% in the livers obtained from rabbits fasted for 18–24 hr.

(*C*) *Microsome studies.* The microsomes demonstrated significant differences in incorporating ability. Control incorporation studies were done with hepatic microsomes obtained from fed animals whose livers were removed at the same time the perfusion study was terminated. These nonperfused livers also supplied the cell sap for all studies. Thus, only the microsomes differed in each combined study, and differences in incorporation could not be ascribed to differences in specific activities due to change in the amino acid levels in the cell sap.

Microsomes derived directly from livers obtained from intact fasted rabbits incorporated 32% less leucine-^{3}H/mg of microsomal RNA compared with microsomes from nonperfused livers supplied by fed rabbits. Hepatic microsomes from perfused livers whose donors were fasting animals incorporated 54% less activity than microsomes from perfused livers obtained from fed rabbits ($P < 0.02$). Microsomes isolated from control and perfused livers supplied by fed rabbits differed by only 16% (Table III). More prolonged incubation failed to increase incorporation in either group.

TABLE II
Albumin Synthesis in the Perfused Liver from Fed and Fasted (18–24 hr) Rabbits

	Fasted albumin synthesis	Portal flow	Fed albumin synthesis	Portal flow
	mg/100 g per 2.5 hr	*ml/g per min*	*mg/100 g per 2.5 hr*	*ml/g per min*
	18.7	1.1	38.0	1.0
	12.7	1.0	44.0	1.2
	13.8	1.1	23.6	1.4
	14.0	0.3–0.35	40.9	0.3–0.35
	15.8	0.3–0.35	34.1	0.3–0.35
	11.4	0.3–0.35	30.6	0.3–0.35
	18.3	0.3–0.35	26.1	0.3–0.35
	22.0	0.3–0.35	29.5	0.3–0.35
			32.1	0.3–0.35
			36.8	0.3–0.35
Mean	15.8		33.7	
±SE*	1.3		2.1	
P value			< 0.001	

The hematocrit value ranged between 24.8–26.9% in all runs and the perfusate albumin 2.7 g/100 ml; range from 2.6–2.9 g/100 ml.
* SE, standard error of the mean.

TABLE III
*Microsome Incorporation with Leucine–^{3}H(Studies–11)**

	Fed		Fasted	
	Non-perfused	Perfused	Non-perfused	Perfused
	cpm/mg of microsomal RNA			
Mean	12,800	10,700	8700	5850
±SE‡	1700	1700	400	300
Per cent change		−16	−32	−54
P value		NS§	<0.05	<0.01

* No. of studies.
‡ SE, standard error of mean.
§ NS, Not significant.

With phenylalanine-^{14}C, hepatic microsomes derived from fed and fasted rabbits were compared with those derived from perfused livers obtained from fed and fasted animals and the response to poly U stimulation was studied (Table IV). Again there was a slight decrease in the microsomal incorporation of phenylalanine-^{14}C in the perfused group compared with nonperfused group under the same experimental conditions. Microsomes from livers obtained from fasted rabbits incorporated 35–41% less phenylalanine-^{14}C than did those derived from livers taken from rabbits that had been fed (Table IV). The addition of polyuridylic acid stimulated phenylalanine incorporation in all groups with a greater effect present in microsomes from perfused livers, which suggests that some polysome disaggregation occurs during perfusion.

DISCUSSION

The introduction of the carbonate-^{14}C technique by Swick, Reeve, and McFarlane (8–10) has made it possible to measure hepatic synthesis of albumin directly by endogenously labeling with ^{14}C over a period short enough to make reutilization unimportant and a prolonged steady-state condition not essential. However there are certain assumptions in the method which have not been clearly proven (30). The rate of albumin and urea synthesis must be constant during the period of measurement, the ^{14}C label must truly be a pulse label so that the liver is rapidly freed of labeled precursor, permitting the fall in specific activity of urea to represent synthesis of unlabeled urea,

TABLE IV
*Microsomal Incorporation with Phenylalanine-^{14}C (Studies–6)**

	Fed		Fasted	
	Nonperfused	Perfused	Nonperfused	Perfused
	cpm/mg of microsomal RNA			
Mean	4630	3715		
±SE‡	200	105	–	–
Per cent change		−20		
Mean	13,500		8795	7920
±SE	540	–	420	150
Per cent change			−35	−41
P value		0.05	<0.01	< 0.01

Phenylalanine −^{14}C + 400 μg polyuridylic acid

	Control	poly-U	Control	poly-U	Control	poly-U	Control	poly-U
Mean	4630	7060	3715	9950	13,500	23,850	7920	18,400
±SE	200	60	105	500	540	750	150	550
Per cent change		+58		+171		+86		+133

* No. of studies.
‡ Standard error of the mean.

and that urea and the guanido carbon of albumin be derived from a common arginine pool (30).

Although there is no reason to expect the rates of synthesis of urea or albumin to remain constant, if the period of measurement is short the chance of significant changes will be minimized. When albumin-^{131}I and carbonate-^{14}C have been used together, good agreement has been reported even though the former label only measures degradation. These comparisons are of course only valid in the steady state (15). In the present study the fractional rates of urea synthesis measured 24 hr apart by endogenous and exogenous urea were not significantly different, and averaged 294 and 286%/day, respectively. Furthermore, when the liver was removed from a rabbit, whose urea disappearance slope was slow (150%/day), 5 hr after carbonate-^{14}C injection, the hepatic arginine guanido-^{14}C specific activity was less than 2% of that found in urea-^{14}C at the same time, indicating that significant persistance of the label within the liver was not occurring. Although McFarlane has reported significant differences in the urea curves after carbonate injection when compared with urea-^{13}C curves in some animals (15), the results of the present study suggest that this is not a major problem. Finally, whereas other pathways for urea synthesis have been suggested, the evidence to support this idea is lacking and the hepatic ornithine-arginine-urea cycle may be considered the only pathway (30–33).

In the present study it would have been possible to use a second injection of urea-^{14}C to measure the fractional rate of urea synthesis in all in vivo studies, but this meant that the fast would have had to be prolonged; thus the endogenous label was preferred.

The changes seen in the whole animal are not unexpected, for fasting or deficient diets have been studied before and albumin production is uniformly lowered (1–3). However, the rapidity with which this occurs was not expected. Recently Kirsch, Frith, Black, and Hoffenberg (4) have shown that the decrease in albumin synthesis in the rat requires days of protein depletion, but supplementation returns albumin synthesis to a value above the control level after only 1 day. Also Staehelin, Verney, and Sidransky have shown that the redistribution of polysomal particles from light to heavy can occur after only 30 min of exposure to a caesin hydrolysate supplemented with tryptophan (5, 34). The results of

this present study show that the effect of fasting is rapid and the lowering of albumin production can be seen after only 18 hr.

The isolated, perfused rabbit liver is a suitable preparation for the study of protein production. With this preparation, as well as with other isolated systems, it is possible to eliminate many of the unknown in vivo regulatory mechanisms that alter hepatic protein production.

In using the perfused liver over a short period of time, the question of synthesis vs. release becomes important. In studies reported by McFarlane (10), albumin guanido carbon specific activity after carbonate injection appears to reach a plateau between 2.5–3 hr. Our results are in accord, for the increase in albumin guanido carbon specific activity during the last 50 min of perfusion was only 14%. While some radioactive albumin still may have remained within the hepatic cells, this quantity was probably small.

The exposure of the perfused liver taken from a fasted animal to the same blood amino acid mixture used for the liver taken from a fed animal failed to reverse the fasting induced decrease in albumin synthesis. It is recognized that the amino acid levels in the perfusate probably represent postabsorptive levels. Nevertheless, perfused livers from fed animals demonstrated a greater synthetic capacity than did the livers from fasted animals.[2]

An increased capacity was also seen when amino acid incorporation by hepatic microsomes were studied. It should be pointed out that only alkali stable–TCA precipitable material was studied; albumin was not isolated in these studies. In this system, the cell sap was derived from livers of fed animals and thus the differences in amino acid incorporation by hepatic microsomes from fed and fasted rabbits was not due to differences in amino acid levels or the medium presented to the microsomes. The data of the present report show that microsomes taken from fasted animals demonstrate a decreased amino acid incorporation into protein whether the donor liver was perfused or intact. A basic alteration in the polysome size has been shown to occur with fasting and only very high levels of amino acids may prevent the disaggregation of the polysome to the free ribosome type in vivo and in vitro (35, 36). The change back to the heavy type of polysome occurs rapidly in the whole animal after feeding (5). Following the ingestion of food, an excess of amino acids must be delivered to the liver and a rapid return to a productive state in terms of protein synthesis could follow.

It was demonstrated that perfusion per se did not impair the amino acid–incorporating ability of hepatic microsomes to any great degree, at least with respect to leucine-^{3}H and phenylalanine-^{14}C. Polyuridylic acid stimulated phenylalanine-^{14}C incorporation in all groups of microsomes, and to the same degree in hepatic microsomes from fed and fasted animals. This observation would imply the same available fraction of functioning ribosomal units was available to stimulation in both groups. However, the hepatic microsomes from perfused livers were stimulated to a greater degree. This latter finding could be explained by a decreased formation of mRNA during perfusion with the greater availability of free ribosomes, but a relative increase in ribosome quantity cannot be excluded. Our data do not differentiate between these possibilities. Staehelin et al. have shown that hepatic polyribosomes obtained from rats fasted overnight who were then force-fed 30 min before sacrifice incorporated more leucine-^{14}C than did the fasted controls, despite Actinomycin D inhibition of mRNA synthesis (5). The increased protein synthesis seen in the force-fed rats was ascribed to a shift in the hepatic polysomes to heavier aggregates. However, while shifts in polysome aggregation following a fast were also observed by Wilson and Hoagland (37), these authors also found that 36% of the liver polysomes are quite stable ($t_{\frac{1}{2}} = 80$ hr) and that a major fraction of these polysomes synthesize albumin (38). It is possible that the presence of a stable albumin polysome may be the basis for the rapid increase in albumin synthesis seen in the refeeding experiments.

[2] For purposes of comparison, the results of the perfusion studies were expressed in the same terms as the in vivo studies. Making an assumption that the delay in release of labeled albumin is ½ hr (10), the control value averaged 157 mg/kg donor rabbit per day and the liver from the fasted preparation 57 mg/kg donor rabbit per day. The liver weights averaged 3.0 and 3.7% body wt in the fasted and fed group, respectively.

Since the perfused liver is without hormonal regulation and the perfusate level of amino acids not elevated, the finding of a lower in vitro synthesis rate for albumin than that observed in vivo is not unexpected.

Further speculation is not rewarding on the exact mechanism underlying the low albumin synthesis rate seen after a short-term fast, but it is clear that a microsomal change is caused by fasting. The effects of even a short-term fast are directed or transmitted to the microsomes which appear to remain inhibited for hours in vitro after the fast has ended, in terms of amino acid incorporation into all protein. These results imply that even the overnight removal of food may cause a diminished protein productive capacity, and suggest hepatic protein and albumin production may not be continuous but may change rapidly, returning to normal production only after food ingestion and the resulting increased amino acid concentration in portal blood.

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