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Effect of Protein Reserves on Liver Regeneration After Partial Hepatectomy. (29690)

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It is well known that animals can maintain nitrogen balance on widely varying intakes of nitrogen(1). If an animal on a high protein diet is transferred to one low in protein content, nitrogen excretion gradually diminishes until, if the intake is not below a certain minimum, equilibrium is again reached. On the other hand, if an animal being maintained on a low or poor quality protein regime is transferred to a diet containing a higher level or better quality protein, initially a period of nitrogen retention occurs which is followed by rising excretion until nitrogen equilibrium is again established. This retained or easily lost nitrogen, is generally considered to represent the more labile protein in the body and is referred to collectively as "protein reserves."

The importance of protein reserves has been discussed elsewhere(2). One of the attributes of a reserve supply of any material is to furnish the substance in question in time of need. One such time is the need for amino acids by the regenerating liver after partial hepatectomy. The experiments to be reported here were designed to learn whether the state of the animals' protein reserves prior to removal of ⅔ of the liver would affect the subsequent liver regeneration taking place during the feeding of a protein free diet.

Methods. Two experiments were carried out. In the first, adult Charles River C-D male rats, weighing approximately 300 g, were placed on a protein-free diet for 2 weeks and then divided into 4 groups of 12 rats each which were fed respectively diets of (1) wheat gluten, (2) gluten + lysine monohydrochloride, (3) gluten + lysine monohydrochloride + threonine or (4) egg white for 1 week. (The nitrogenous part of each diet was added at the expense of corn starch to give 1.60% nitrogen in the diet.) The composition of these diets is shown in Table I. After this 7-day repletion period, one-half of the rats receiving each diet were subjected to partial hepatectomy by the method of Higgins and Anderson(3) and all groups were again placed on the protein-free diet. Seven days later, both operated and unoperated rats were anesthetized with nembutal and blood samples taken by heart puncture. The animals were then killed and the livers removed and weighed.

A second experiment was carried out in the same general manner, except that slightly smaller rats were used (average wt. 225 g), the group size was increased from 12 to 20

TABLE I. Composition of Diets (g/100 g Diet).

	1	2	3	4
Salt mixture USP XIV	4.0	4.0	4.0	4.0
Cellu-flour	5.0	5.0	5.0	5.0
Corn oil	10.0	10.0	10.0	10.0
Vitamin mix*	1.0	1.0	1.0	1.0
Wheat gluten†	11.1	11.1	11.1	—
Dried egg white‡	—	—	—	11.7
L-Lysine monohydrochloride	—	.35	.35	—
L-Threonine	—	—	.10	—
Corn starch	68.9	68.5	68.4	68.3

* Furnishing the following micronutrients per 100 g of food: In mg—thiamine, 1.0; riboflavin, 2.0; pyridoxine, 1.0; calcium pantothenate, 10.0; niacinamide, 10.0; inositol, 5.0; choline, 100.0; p-aminobenzoic acid, 30.0; biotin, 0.05; folic acid, 0.2; α-tocopherol, 14.2; menadione, 14.2; B_{12} triturate (0.1% trituration with mannitol), 10.0. In units—calciferol, 300; vitamin A palmitate, 1600.

† General Mills Pro-80 Wheat Gluten.

‡ Obtained from Henningsen Bros., N. Y.

TABLE II. Body Weight Changes Before and After Partial Hepatectomy (Exp 1).

	Diet	Pre-surgery* wt gain (g)	Post-surgery† wt loss (g)	Final body wt (g)
Partially hepatecto-mized	Gluten	15.4	20.3	232.4
	Gluten + lysine	49.9	34.0	253.7
	Gluten + lysine + threonine	67.5	43.5	262.5
	Egg white	88.8	46.5	280.6
Unoperated	Gluten	15.7	19.4	233.8
	Gluten + lysine	54.0	32.2	260.5
	Gluten + lysine + threonine	69.3	42.2	264.8
	Egg white	84.5	39.1	284.0

* Avg weight of all groups after the initial 2-wk depletion period and prior to repletion was 238 g. Animals partially hepatectomized after 7 days repletion.

† Weight losses occurring on a protein-free diet. For the unoperated controls the "post-surgery" weight losses are those taking place during the time corresponding to the regeneration period for the operated rats.

rats and a 3-day instead of 7-day regeneration period was allowed. The diets employed in Experiment 2 were (1) gluten, (3) gluten + lysine monohydrochloride + threonine or (4) egg white (Table I). For both experiments the weight of liver remaining after surgery ("residual" liver) was calculated from the weight of liver removed assuming the percentage of liver removed in the operation to approximate that removed in a preliminary experiment with 6 rats fed wheat gluten and 6 fed egg white. The procedure was found to remove 66.3 ± 1.8%* of the liver with no significant difference between these 2 groups.

Total protein was determined on heparinized plasma using a Technicon autoanalyzer and the biuret method(4).

Results. The changes in body weight taking place during the course of Experiment 1 are shown in Table II. During 2 weeks on the protein-free diet, the rats lost approximately 60 g or 20% of body weight. The weight gains occurring during the repletion period became progressively greater as the quality of the dietary protein was improved. The animals repleted with gluten gained 15 g, while those fed egg gained 86 g (Table II). In the unoperated rats, weight losses during the second depletion period, as one would expect, were greater in the animals having previously ingested the egg diet than in those fed gluten. However, the loss as a percentage of the previous gain was much less for the former with the consequence that after the 7-day depletion period, the gluten-fed rats were 50 g lighter than those previously fed egg. Body weights of the partially hepatectomized rats were not greatly lower than those of the controls. Weight of liver tissue removed ranged from 6 to 10 g or 2 to 3 g dry weight. The difference in body weight between operated and unoperated rats was approximately equal to the dry weight of liver removed (Table II). These data suggest that food consumption was not depressed in the partially hepatectomized rats as compared to controls.

* Standard error of mean.

The liver regeneration data are shown in Table III. Although final liver weights are greater in the rats having been fed egg as compared to those that ingested gluten, as a percentage of the weights of the unoperated controls, there were no consistent differences in regeneration as a result of diet prior to partial hepatectomy. Also, although the size of the liver remnant in the animals fed egg white was almost twice that in those fed gluten, the weight of new liver tissue formed was approximately the same in all groups (Table III).

In the second experiment, slightly smaller rats were used with a 3-day rather than 7-day regeneration period. The liver weights resulting from the shorter regeneration time are also shown in Table III. Again, no dietary effect on the percentage of liver regeneration was seen and the weight of new liver tissue formed was approximately the same in all groups, in spite of an almost 2-fold variation in weight of the liver remnant.

TABLE III. Effect of Pre-Treatment Diet on Liver Regeneration on a Protein-Free Diet.

Pre-treatment diet	Final liver wt (g) A Operated groups	Final liver wt (g) B Unoperated groups	Regeneration index A/B × 100	C Residual liver wt (g)	Wt liver formed (g) A − C
		Experiment 1*			
Gluten	6.2	7.8	79	3.0	3.2
Gluten + lysine	7.1	8.7	82	3.7	3.4
Gluten + lysine + threonine	7.2	8.7	83	4.1	3.1
Egg white	7.6	9.9	77	5.0	2.7
		Experiment 2†			
Gluten	4.3	6.0	72	2.4	1.9
Gluten + lysine + threonine	5.0	6.5	77	3.2	1.8
Egg white	5.7	7.8	73	3.7	1.9

* 7 Days regeneration. † 3 Days regeneration.

Plasma protein concentrations at completion of Exp. 1 and 2 are shown in Table IV. These data have been subjected to an analysis of variance as described by Snedecor(5). In both experiments plasma protein concentrations of the rats subjected to partial hepatectomy were significantly lower than those of the unoperated rats ($P<.01$). Also in both experiments the final plasma protein concentrations in the rats fed egg white prior to the final depletion period were significantly greater than those fed wheat gluten during this period, both for operated and unoperated rats ($P<.05$). In Exp. 1, the unoperated rats previously fed gluten + lysine + threonine had a lower average plasma protein concentration than those previously fed gluten alone. This was not the case for the partially hepatectomized animals nor was it observed in Exp. 2 among either the operated or unoperated rats. The significance of this observation is not clear and more data are required.

TABLE IV. Final Plasma Protein Concentrations (g/100 ml).

Pre-treatment diet	Operated	Unoperated
	Experiment 1†	
Gluten	4.45 ±.19*	5.49 ±.09
Gluten + lysine	4.80 ±.23	5.30 ±.04
Gluten + lysine + threonine	4.80 ±.07	5.08 ±.12
Egg white	5.20 ±.19	5.78 ±.11
	Experiment 2‡	
Gluten	4.43 ±.18	5.16 ±.11
Gluten + lysine + threonine	4.42 ±.09	5.26 ±.10
Egg white	4.95 ±.14	5.67 ±.06

* Mean ± S.E.
† 7 Days regeneration on a protein-free diet.
‡ 3 Days regeneration on a protein-free diet.

The effects of the partial hepatectomy and low level of protein reserves were additive and the lowest plasma protein levels were found in the partially hepatectomized rats previously fed wheat gluten. It should be emphasized that these differences in plasma protein concentration persisted 3 to 7 days after all rats had been placed on a protein-free diet. The survival data, although limited, also show an additive effect of previous dietary history and partial liver resection. Only 3 rats died during the recovery period and all 3 had been on wheat gluten prior to surgery, 1 of 6 in Exp. 1, and 2 of 10 in Exp. 2.

Discussion. It was demonstrated many years ago by Vars and coworkers that the regeneration of rat liver after partial hepatectomy can effectively be accomplished in the absence of dietary protein(6,7). These workers also demonstrated that furnishing dietary protein resulted in superior regeneration. Similar results have been reported for lysine-deficient rats by Lavers *et al*(8). The amino acids required are furnished by other tissues of which muscle is probably the most important(9). In the present experiments the point investigated was whether or not liver regeneration on a protein-free diet would be improved by the existence of fuller protein stores at the time of operation. The results show no such effect, indicating that control of the formation of new liver tissue is not limited by the total supply of amino acids moving from muscle and other tissues to the liver through the plasma. However, the plasma protein data suggest that the rats with fuller protein stores were better able to withstand the stress of partial liver resection and this is supported by the limited survival data. The precedence given to cellular protein synthesis in regenerating liver over plasma protein synthesis has previously been reported by Braun *et al*(9).

It seems pertinent to draw attention to the possible relevance of these observations to problems in human nutrition. It has been pointed out by Scrimshaw and Behar(10) that in areas where kwashiorkor is common, an acute episode of this childhood protein deficiency condition is usually precipitated by the stress of infection. Arroyave(11) has reported that in such areas children not suffering from kwashiorkor have the same low percentage of lean body mass as children suffering from this disease, indicating that a large percentage of the children are protein-depleted. The loss of nitrogen associated with the stress of infection and concomitant diarrhea precipitates the acute protein deprivation disease, kwashiorkor, with symptoms, among others, of severe hypoproteinemia and edema. The lowered resistance to infection in such children has been ascribed to their protein-depleted state(10). In the analogy being drawn, the liver nitrogen removed by partial liver resection represents the infection-induced loss of body nitrogen in a severely protein-depleted organism.

Summary. The regeneration of rat liver after partial hepatectomy in rats fed a protein-free diet has been used in an attempt to assess the importance of protein reserves. Groups of protein-depleted adult male rats were fed diets of varying protein quality for one week, and then subjected to removal of two-thirds of the liver. Recovery periods of 3 and 7 days were studied during which time a protein-free diet was fed. No effect of the previous dietary history on the weight of new liver tissue formed was observed. Plasma protein levels were significantly lower after 3 and 7 days protein-depletion in animals previously fed gluten as compared with those fed egg. Levels also were significantly depressed as a result of the partial hepatectomy, and the effects of poor quality dietary protein and liver resection were additive. It is suggested that under the conditions investigated, the existence of fuller protein stores was of physiological advantage to the organism and possibly of survival value.

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