Piecemeal necrosis, particularly accentuated on the lobular periphery near the portal tracts, leads to a relentless elimination of all liver cells and their replacement by immunologically active cells within 20 days. The initial stage of this rejection resembles the peripheral piecemeal necrosis of chronic active hepatitis and progressing cirrhosis in man.

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Metabolic Adaptations in Higher Animals. VII. Responses of Glutamate-Oxalacetate and Glutamate-Pyruvate Transaminases to Diet.* (28164)

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Freedland and Harper(1) found that hepatic glucose-6-phosphatase (G-6-Pase) activity of rats, fed a diet in which most of the carbohydrate (dextrin)† was replaced by protein or fat, increased within 24 to 48 hours, then gradually returned to normal. They suggested that the immediate rise in G-6-Pase activity might represent a primary adaptation to low glucose intake and that the return of G-6-Pase activity to control level might be accompanied by secondary adaptations, such as an increase in rate of utilization of amino acids or fatty acids for energy, which could reduce the need for synthesis of glucose and, hence, for elevated G-6-Pase activity.

If transaminase activity increased before G-6-Pase activity returned to normal in rats fed diets high in protein and low in glucose, as would appear to be the case from observations of Rosen *et al.*(2), such a response could be considered a secondary adaptation of the type postulated. Therefore, the activities of some transaminases in organs from rats fed high-protein diets for different periods of time were studied.

Methods. Male Holtzman rats which had been maintained on a stock diet were fed the appropriate control diet for at least 3 days before they were used in an experiment. The composition of diets used most frequently is summarized in Table I.

At the times indicated in the Tables rats were decapitated and organs removed and chilled immediately. About 1 g of liver, kidney or heart was weighed and homogenized with a Teflon pestle in 9 or 19 volumes of distilled water at 0-4°C. Muscle was homogenized in a Duall glass homogenizer (Kontes Glass Co.). Incubations were at

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† The term "dextrin" refers to the product obtained when moist cornstarch is heated for 2 hours in an autoclave at 115°C and then dried and ground.

TABLE I. Composition of Diets.

	Control	High-protein No. 1	High-protein No. 2	High-fat No. 1	High-fat No. 2
Casein					
% of diet	25	80	90	36	40
% of calories	23	77	88	22	26
"Dextrin"					
% of diet	65	10	0	16	—*
% of calories	66	11	0	10	
Margarine					
% of diet	—	—	—	38†	49

All diets contained corn oil, 5%; salts(13), 5%; and vitamins(13).

* About 0.5% of the total calories of this diet came from the sucrose used to triturate the water-soluble vitamins.

† Commercial margarine was melted and the fat taken from the top of the melt for use in High-fat diet No. 1. In High-fat diet No. 2, the margarine was used as purchased and considered to be 80% fat.

37°C except for spectrophotometric determinations which were done at ambient temperature. Nitrogen content was determined by the Kjehldahl method with mercuric oxide as the catalyst. In the experiment on the effects of starvation, liver protein was determined with Folin-Ciocalteu phenol reagent (3).

Determination of enzyme activities. The methods used were essentially those described by Karmen *et al.*(4) and Wroblewski and LaDue(5). Rate of reaction was expressed as mmoles/hr/g of liver or per 100 g of rat. The nitrogen content of liver from rats fed high-protein diets was from 100-115% of control value, so expression of activity on a nitrogen basis did not alter the trends observed. The molecular extinction coefficient for DPNH at 340 mμ of 6.2×10^6(6) was used for the calculations. In each series of determinations, the activity of one liver was estimated on several different amounts of homogenate.

In preliminary experiments the spectrophotometric method was used to estimate the activities of both glutamate-oxalacetate (GOT) and glutamate-pyruvate transaminase (GPT). A 2-fold increase in hepatic GPT activity was observed when the casein content of the diet was increased from 25% to 80%. Rosen *et al.*(2) reported that a 4-fold increase was produced by a similar experimental treatment. To investigate this discrepancy a colorimetric method was used for estimation of GOT and GPT(7). The results obtained were similar to those of Rosen *et al.*(2) and were confirmed by manometric measurement of glutamic acid formation(8). The colorimetric method was therefore used for measurement of hepatic GPT activity.

Procedures used which differ from those in the published colorimetric method(7) are described below. L-aspartic acid and L-alanine were used as substrates. When the method was used to estimate hepatic activities, 0.03 mmole of α-ketoglutarate and 0.14 mmole of an amino acid were added per 1.2 ml of reaction mixture. Incubations were at 37 $\pm$ 1°C. Oxalacetate formed by the reaction catalyzed by GOT was decarboxylated with aniline citrate(9). Two reaction mixtures were prepared from each homogenate; one contained at least twice as much tissue as the other.

No increase in activity of either enzyme resulted from incubation of the homogenates with pyridoxal phosphate under the conditions described by Meister(10). Mixing of liver homogenates from rats fed 2 different levels of casein resulted in enzyme activities close to those predicted and did not indicate the presence of activators or inhibitors.

Results. Glutamate-oxalacetate transaminase. The activity of glutamate-oxalacetate transaminase (GOT) per g of liver increased with increasing casein intake. The values, expressed as mmoles product/hr/100 g body weight, for rats fed for 7 days on diets containing 0, 11, 25 and 50% casein were, respectively, 24, 38, 45 and 63. Increasing the casein content of the diet to 75 or 90% did not further increase GOT activity. GOT activities expressed per g fresh liver showed the same trend. Average food intake per day of the group fed the 75% casein diet was 13 g, and of the group fed the protein-free diet, 14 g.

GOT activity per g of liver from rats fed an 80% casein diet for 1, 23, or 100 days was about 180% of control value (Table II). Similar but less rapid increases were obtained after feeding a 90% casein diet (Table III) or a diet containing 80% of egg white. The

TABLE II. Effect of Age on Response of Hepatic GOT and GPT to High-Casein Intake.

No. of rats	Days of feeding	Enzyme activity — GOT — Per g liver	Per 100 g rat	GPT — Per g liver	Per 100 g rat
			(mmoles product/hr)		
25% Casein Diet					
6	1 to 23	6 ± .3†	26	1.0 ± .1	4
5	100	7 ± 1	28	5.1 ± .9	21
4	310	8 ± .5	24	2.3 ± .1	7
80% Casein Diet					
4	1	11 ± .5	51	1.4 ± .1	6
4	2	10 ± .7	54	2.2 ± .2	12
4	3	12 ± .7	73	3.0 ± .2	20
4	4	10 ± 1.1	62	5.0 ± .5	31
4	5	15 ± .5	93	5.6 ± .3	35
4	8	13 ± .3	83	5.2 ± .2	33
4	23	12 ± .9	60	6.3 ± .5	31
9	100	12 ± 1	49	8.6 ± .8	35
4	310	9 ± .4	22	4.8 ± .1	12

* Rats ranged in weight from 90 to 130 g on days 1 through 8 and from 350 to 480 g by 100 days.
† Mean ± standard error.

TABLE III. Effect of Pair-Feeding and of Feeding a 90% Casein Diet on Hepatic GOT Activity.

Diet	Days of feeding	Enzyme activity — Per g liver	Per 100 g rat
		(mmoles product/hr)	
25% Casein	1	3*	17
	2	3	16
	5	4	16
	14	5	23
25% Casein pair-fed†	1	4	16
	2	6	23
	5	5	21
90% Casein	1	4	21
	2	5	28
	5	7	41
	14	9	52

* Mean of values from 6 rats.
† Food intake of pair-fed rats was limited to amounts eaten by similar rats fed the high-protein diet.

increase in GOT activity in livers of rats fed the 80% casein diet was about the same whether the rats used were 1 or 4 months old. However, only small increases were observed if rats 10 or 14 months old were used (Tables II and IV). An increase in hepatic GOT activity as a result of feeding a high-protein diet has recently been reported by Schimke(11).

Limited food intake produced a comparatively small increase in total GOT activity per 100 g of rat (Tables III, IV and V). Total hepatic GOT activity decreased in rats starved for 5 days (Table VI).

Glutamic-pyruvate transaminase. The maximum response of GPT to the 80% casein diet, an increase equal to 400% of control value, was observed only in livers from rats fed the high-casein diet for at least 4 days (Table II). A similar increase was obtained by feeding a diet containing 80% of egg white. The increases observed were similar to those reported by Rosen *et al.*(2).

The results in Table II show that hepatic GPT activity was higher in livers from control rats 10 months old than in young rats.

TABLE IV. Effects of Feeding a High-Protein Diet for 8 or 9 Days on Hepatic GOT and GPT Activities.

Diet	Enzyme activity — GOT — Per g liver*	Per 100 g rat	GPT — Per g liver	Per 100 g rat
Rats 5 mo old		(mmoles product/hr)		
25% Casein	4 ± .2†	10	2 ± .1†	7
25% Casein pair-fed‡	4 ± .2	10	4 ± .7	10
80% Casein	6 ± .6	20	6 ± .7	19
Rats 14 mo old				
25% Casein	6	16	2	6
80% "	6	21	3	9

* Activity was measured spectrophotometrically at room temperature; activities in Tables II and III were measured at 37°C.
† Mean ± standard error.
‡ Food intake of pair-fed rats was limited to amounts eaten by similar rats fed the high-protein diet.

TABLE V. Effect of Food Restriction on Hepatic GOT and GPT Activities of Rats Fed a 25% Casein Diet.

No. of rats	Wt of rats (g)	Enzyme activities GOT Per g liver	GOT Per 100 g rat	GPT Per g liver	GPT Per 100 g rat
		(% of control values)			
2	100	130	110	110	100
2	100	200	130	110	80
5	200	110	100	80	70
4	200	130	90	120	90
3†	100			125	100

* The rats were fed for 2 or 3 days amounts of the 25% casein diet that allowed only weight maintenance. Control values were for rats fed the 25% casein diet *ad libitum*.
† These rats were pair-fed for 9 days with others fed the 80% casein diet *ad libitum*.

Similar observations have been reported previously(12). Some very high values were obtained for the control rats in the 100-day experiment, resulting in a high average activity with a large standard error. After this observation was made the activity in livers of several more control rats of similar age was estimated; no values exceeding 3.0 mmoles per g were obtained.

The response of GPT activity to increased casein intake was smaller for rats fed the experimental diets for 10 months and for 14-month-old rats fed these diets for 8 or 9 days (Tables II and IV).

Restriction of food intake resulted in no change or a small increase in GPT activity (Tables IV and V). In contrast, rats starved for 5 days had 60% more total GPT activity and 40% less total liver protein than fed rats (Table VI).

TABLE VI. Effect of 5 Days of Starvation on Hepatic GPT and GOT Activity.

Gross changes	Fed stock diet	Starved
Final wt rats	187	102
Final wt livers	8.2	3.2
g nitrogen × 6.25/liver	1.4	.6
g nitrogen × 6.25/100 g liver	17.4	19.1
GPT activity		
mmoles pyruvate/g liver/hr	2.0	8.5
mmoles pyruvate/liver/hr	17 ±.4*	27 ± 3
GOT activity		
mmoles oxalacetate/g liver/hr	3.8	7.7
mmoles oxalacetate/liver/hr	31 ±.5	24 ±.4
μmoles oxalacetate/ml serum/hr†	3.2 ±.1	7.2 ±.6

* Mean ± standard error of values for 6 rats weighing 155 g at beginning of experiment.
† Fed and starved rats had the same amount of GPT activity per ml of serum.

Activities of GOT and GPT in other tissues and in serum. The activities of these enzymes have been reported to increase in the serum under conditions involving tissue destruction. Therefore, these activities were estimated in the serum of the starved rats. GOT activity per ml of serum from starved rats was 225% of that for the controls (Table VI). GPT activity per ml of serum was the same in the 2 groups. Small increases, 10% or less, in activities of these 2 enzymes in sera of rats fed diets deficient in protein were observed. Neither of the enzyme activities increased in sera from rats fed the 80% casein diet.

The activities of GOT and GPT in gastrocnemius muscle, heart, and kidney were estimated. Activities in these tissues were not altered in rats fed a high-protein diet.

Effect of diets low in carbohydrate on activities of GOT and GPT. The high-protein diets used in these studies were necessarily low in carbohydrate. To determine whether the high-casein or low-carbohydrate intake was responsible for the response of GOT and GPT to the high-casein diet, diets which were high in fat, low in carbohydrate and adequate in protein were fed to 3 groups of rats (Table VII); 3 control groups were fed the control diet containing 25% of casein and 5% of fat. There were no marked changes in hepatic GPT or GOT activities of rats fed a high-fat diet. The rats fed the high-fat diets had total and relative liver weights similar to those of the control rats and gained weight slightly more rapidly.

Discussion. Rosen *et al.*(2) have shown

TABLE VII. Effect of Diets High in Fat and Low in Carbohydrate on Hepatic GOT and GPT Activities.

Diet	Days of feeding	Enzyme activity GOT	GPT
		(mmoles product/g liver/hr at 37°C)	
Exp 1	8		
25% casein, low fat		5 ± .4*	1 ± .1
High-fat No. 1		5 ± .5	1 ± .1
Exp 2	7		
25% casein, low fat		6 ± .3	2 ± .2
High-fat No. 2		7 ± .4	2 ± .2
Exp 3	24		
25% casein, low fat†		7	.8
High-fat No. 2		6 ± .1	.8 ± .1

* Mean ± standard error for 5 or 6 rats weighing about 150 g.
† 2 rats.

that GPT activity increases in livers of rats subjected to several treatments (cortisol, alloxan, starvation) which stimulate gluconeogenesis. However, the observation that GPT activity increases as dietary casein content increases from 10-40%(2) while carbohydrate intake is presumably adequate, and that it does not increase in rats fed a high-fat, low-carbohydrate diet, indicates that the enzyme responds to increased casein intake, not to decreased carbohydrate intake. The data available indicate that the activities of these 2 transaminases increase as protein catabolism increases.

An increase in the activity of an enzyme involved in amino acid catabolism within 3 days after feeding a high-protein diet, with the activity remaining elevated as long as the protein intake remains high, could represent part of a secondary adaptation of the type postulated to precede a return of G-6-Pase activity to normal in rats fed a high-protein diet(1). The observed responses of GOT and GPT fulfill these conditions. However, since the transaminases increase as the dietary casein content increases from 10-40% while G-6-Pase does not, it is evident that the stimuli responsible for the elevations in transaminase and G-6-Pase activity are different.

Summary. The activities of glutamic-oxalacetic (GOT) and glutamic-pyruvic (GPT) transaminases have been estimated in livers from rats subjected to limited food intake or fed diets high in casein or fat. Increases in GOT and GPT activity were observed in livers from rats fed an 80% casein diet; the responses were greater in young than in old rats. Increases in GOT and GPT activity due to limited food intake were much smaller than those resulting from feeding a high-protein diet. Feeding low-carbohydrate, high-fat diets caused no observable change in the activity of either enzyme.

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