TABLE I. Spectrophotofluorimetric Determinations Showing Distribution of Dye Recovered 30 Minutes After Injection.

Organ	% transmission
Intestinal contents	49.5
Liver	37.1
Kidneys	4.9
Spleen	1.2
Lungs	1.6
Intestinal wall	4.6

film using a yellow-green filter with a U.V. lamp as the only source of illumination. The fate of intravenously administered fat emulsions has been widely considered in view of the use of such emulsion for intravenous administration(1). The clearance mechanism has been considered by other workers and the majority implicate the reticuloendothelial system. The high concentration of dye in the liver and the exponential disappearance from the blood stream are in accord with their view. However, presumptive blocking of fat uptake by the reticuloendothelial cells with such agents as carbon black, causes no significant change in rate of removal from the blood(4).

The appearance of the dye (and hence fat) in the duodenum, especially so rapidly after injection, is surprising if one considers the process to involve phagocytosis and subsequent transport systems. These results are interesting in view of the observation by Juhlin(5), that spheres of polymethylmethacrylate are also transported to the bile within minutes after injection into rabbits. Juhlin found a dependence upon particle size, large particles (0.2-0.8 μ diam.) rarely entering the bile and smaller particles (0.02-0.11 μ diam.) entering rapidly. In the present work, the particle size, though not accurately determined, is of the order of 0.2-0.7 μ diam.(6). In the preparation of fat emulsions for intravenous feeding it has generally been assumed that the particle size should be as small as possible. In view of the above findings this view may need to be considerably modified (1).

Summary. Oil emulsions containing a fluorescent dye were given intravenously to rats. Rate of removal from the blood stream was typical for reticuloendothelial clearance of a colloid. The emulsion accumulated in the liver but appeared in the bile duct and duodenum within minutes after injection. Approximately one-half of the dye was found in the intestinal contents.

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1. Geyer, R. P., *Physiol. Rev.*, 1960, v40, 150.

2. Benacerraf, B., Biozzi, A., Halpern, B. N., Stiffel, C., *Physiopathology of the Reticuloendothelial System.* Blackwell Scientific Publications, Oxford, 1957, p52.

3. Lorver, S. H., Shay, H., *Gastroenterology,* 1952, v2, 262.

4. Waddell, W. R., Geyer, R. P., Clarke, E., Stone, F. J., *Am. J. Physiol.,* 1954, v177, 90.

5. Juhlin, L., *Acta Physiol. Scand.,* 1960, v49, 224.

6. Pinter, G. C., Zilversmit, D. B., *Fed. Proc.,* 1961, v20, 275.

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Metabolic Adaptations in Higher Animals. VIII. Effect of Diet on Ornithine-α-Ketoglutarate Transaminase.* (28220)

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Glutamate-oxalacetate (GOT) and glutamate-pyruvate transaminase (GPT) activities are affected by dietary and hormonal treatments(1-3). Several other transaminases have been identified in mammalian tissues (4-10) but there have been few studies of effects of diet on them.

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The observations reported here arose from a study of the rate of glutamate formation from α-ketoglutarate and various individual amino acids added to homogenates of liver from rats fed moderate or high protein diets. Several amino acids were used as substrates in preliminary experiments but when ornithine-α-ketoglutarate transaminase activity was found to be elevated in liver from rats fed a high protein diet, effects of diet on this enzyme were examined in more detail.

Experimental procedure. Animals and diets. The care of the rats and preparation of diets were as described(3). The basal diet was an adequate purified diet. When protein or fat content was increased, carbohydrate content was decreased.

Substrates. L-amino acids were used. Preliminary experiments with ornithine-α-ketoglutarate transaminase indicated that the pH optimum was about 8.0. Each amino acid used as a substrate was dissolved in 0.1 M tris (hydroxymethyl) aminomethane at a concentration of 0.8 M; pH was adjusted to 8.0 with HCl. In some of the experiments 0.1 M potassium phosphate buffer was used and the pH of solutions of substrate adjusted to 7.0. α-Ketoglutarate (Sigma Chemical Corp.) was dissolved at a concentration of 0.2 M in the appropriate buffer and the pH of the solution was adjusted with NaOH.

Transamination reaction. 3 ml of amino acid solution and 1 ml of diluted liver homogenate, prepared as described(3), were pipetted into a test tube. Two reaction mixtures were prepared from each homogenate; 1 contained twice as much liver as the other. The reaction was started by addition of 1 ml of α-ketoglutarate solution and allowed to proceed at 37°C ± 1 for 1 hour ± 1 minute. The reaction was stopped by placing the tubes in boiling water for 5 minutes.

Determination of L-glutamate. L-glutamate was determined by a decarboxylation method adapted from that used by Cammarata and Cohen(4). The L-glutamate decarboxylase preparation (Sigma Chemical Corp.) was made from *Escherichia coli* ATCC strain 11246 as described by Gale(11). Each reaction mixture was centrifuged to separate the precipitated protein from the solution, and citric acid was added to bring the solution to pH 5. The Warburg flasks used were flushed and filled with nitrogen(12).

Results. Transamination between α-ketoglutarate and amino acids. In a survey experiment, rates of glutamate formation from α-ketoglutarate and each of several amino acids in the presence of liver homogenates from rats fed either the 80% or the 25% casein diets were measured. The following L-amino acids were used as substrates: methionine, phenylalanine, leucine, isoleucine, valine, and ornithine. Rates of reaction were measured at pH 7 and pH 8. Only with phenylalanine or ornithine as the amino acid substrate was the rate of reaction with α-ketoglutarate rapid enough to be measured by this manometric method. Rate of transamination between phenylalanine or ornithine and α-ketoglutarate was about 0.2 mmole/hour/g of liver from rats fed the 25% casein diet. Only with ornithine as the amino acid substrate was the rate of glutamate formation elevated when the casein content of the diet was increased from 25% to 80%.

Mixing of liver homogenates from rats fed the 2 levels of casein resulted in ornithine transaminase activities which were 105 ± 4% of the predicted value, indicating that the difference between the activities of liver from the 2 groups was not caused by differences in inhibitor or activator concentrations. Preliminary experiments indicated that most of the hepatic ornithine transaminase activity was in the particulate portion of homogenized rat liver. No increase in activity was obtained by homogenizng the liver with a rotating blade homogenizer (VerTis "23," VerTis Co., Inc.) or with a solution of 0.1% of Tween-80 (Atlas Powder Co.).

Throughout this paper the term "ornithine transaminase activity" refers to rate of formation of glutamate from α-ketoglutarate and ornithine in presence of rat liver homogenate.

Effect of a high protein diet on ornithine transaminase activity. The ornithine transaminase activity per g of liver from young rats fed the 80% casein diet for 1 day was over double the control value, and was from 4- to 8-fold the control value in livers from rats fed this diet for 4 days (Table I). A si-

TABLE 1. Effect of High Protein Diet on Hepatic and Renal Ornithine Transaminase Activity.

Days on diet	Ornithine transaminase activity: 25% casein, Per 10 g organ	25% casein, Per 100 g body wt	80% casein, Per 10 g organ	80% casein, Per 100 g body wt
	(mmoles of glutamate/hr)			
Liver				
1	1.6 ±.2*	.6	4.0 ±.4*	2.0
4			8.0 ±.3	4.6
8	.9 ±.2	.4	9.6 ±.2	6.4
20			6.5 ±.5	3.8
210	.9 ±.3	.3	1.9 ±.2	.7
310	.9 ±.3	.3	.8 ±.2	.2
9†	.7	.2	1.9 ±.2	.6
Kidney				
4	1.2	.1	1.3	.17
8			1.2	.17
20			1.1	.15
210	1.0	.05	.9	.07

* Mean ± standard error of values for at least 3 rats.
† 14 mo old.

milar response to a high dietary level of protein was observed when egg white was substituted for casein. The response to protein was much smaller in 14-month-old rats fed the 80% casein diet for 9 days.

Addition of extra Vit. B_6 to the diets did not increase ornithine-transaminase activity in liver from the rats fed the 80% or the 25% casein diets for 4, 8 or 20 days. Also incubation of liver homogenates from rats fed these diets, with pyridoxal phosphate(13), did not affect the activity of the enzyme.

Activity in kidney and muscle. Ornithine-transaminase activity per g of kidney was the same in young and old rats fed either the 25 or 80% casein diet. The kidney activity per 100 g body weight was higher in rats fed the 80% casein diet because of the kidney hypertrophy in these animals (Table I).

Ornithine-α-ketoglutarate transaminase activity is either absent from the gastrocnemius muscle of rats or the activity is very low.

Effect of decreased food intake. When rats were fed the 80% casein diet, their food consumption decreased 30 to 40% during the first week. Ornithine-transaminase activity of liver from control rats pair-fed with those receiving the 80% casein diet was below the value for control rats fed *ad libitum* 1.0 compared to 1.6 mmoles/10 g liver, respectively.

The effect of starvation for 4 days is shown in Table II. Hepatic activity per 100 g of body weight in starved rats was about one-fifth of the value for those fed the 25% casein diet *ad libitum*.

Effect of a diet low in carbohydrate. The 80% casein diet differed from the control diet in both protein and carbohydrate content. To observe the effect of a low carbohydrate intake a group of rats was fed a diet high in fat, low in carbohydrate, and adequate in protein(3) for 3 weeks. Ornithine-transaminase activity in liver was the same whether the rats were fed the high fat or the control (25% casein, low fat) diet (Table III). The growth rates of the two groups were similar.

TABLE II. Activity of Ornithine Transaminase In Liver from Rats Starved for 4 Days.

	Enzyme activity (mmoles glutamate/hr)
Starved rats	
Per 10 g of liver	.6 ± .1*
Per 100 g of rat	.2
Per total liver	.25
Similar rats fed 25% casein diet *ad libitum*	
Per total liver	1.3

* Mean ± standard error of values for 4 rats with avg initial weight of 168 g and final weight of 120 g.

Discussion. Ornithine-transaminase activity in liver increased when rats were fed a high protein diet. This was apparently a response to the high protein intake and not the concomitant low carbohydrate intake, because the even lower carbohydrate intake of

TABLE III. Effect of a Diet High in Fat and Low in Carbohydrate.

Diet	No. of rats	Hepatic ornithine transaminase activity: Per 10 g of liver	Per 100 g of rat
		(mmoles glutamate/hr, 37°C)	
25% casein	4	1.0 ± .3	.35
High fat*	5	1.1 ± .2	.39

The diets were fed for 3 wk. Avg starting weight of the rats was 60 g.

* Contained 40% casein and 49% margarine(3). In control diet 23% and in high fat diet 26% of the calories were from casein.

rats fed a high fat diet did not result in an observable increase in the activity of this enzyme.

The loss of ornithine-transaminase activity was greater than the loss of liver protein in starved rats. This response to starvation is different from that of glutamate-oxalacetate transaminase which was conserved relative to liver protein, and also from that of glutamate-pyruvate transaminase, which increased relative to liver protein(2,3). This and other observations(1,6) indicate that the responses of different transaminases to a single treatment may be quite different.

Summary. The activity of ornithine-transaminase per g of liver from young rats fed an 80% casein diet for 4 days was from 4- to 8-fold the value for a control group fed a 25% casein diet. However, when mature or old rats were used in a similar experiment the response to increased protein intake was smaller or absent. Moderate restriction of food intake decreased the activity of the enzyme slightly, and starvation decreased it markedly. Feeding a low-carbohydrate, high-fat diet adequate in protein did not affect ornithine-transaminase activity.

1. Knox, W. E., Auerbach, V. H., Lin, E. C. C., *Physiol. Rev.*, 1956, v36, 164.

2. Rosen, F., Roberts, N. R., Nichol, C. A., *J. Biol. Chem.*, 1959, v234, 476.

3. Waldorf, M. A., Kirk, M., Linkswiler, H., Harper, A. E., submitted for publication.

4. Cammarata, P. S., Cohen, P. P., *J. Biol. Chem.*, 1950, v187, 439.

5. Rowsell, E. V., *Biochem. J.*, 1956, v64, 235.

6. Lin. E. C. C., Civen, M., Knox, W. E., *J. Biol. Chem.*, 1958, v233, 1183.

7. Hammar, C. H., *Biochem. Z.*, 1961, v333, 486.

8. Quastel, J. H., Witty, R., *Nature*, 1951, v167, 556.

9. Finchham, J. R. S., *Biochem. J.*, 1953, v53, 313.

10. Meister, A., *J. Biol. Chem.*, 1954, v206, 587.

11. Gale, E. F., Glick, D., *Methods of Biochem. Anal.*, New York, 1957, v4, 285.

12. Umbreit, W. W., Burris, R. H., Stauffer, J. F., *Manometric Techniques*, Minneapolis, 3rd ed., 1957, p71.

13. Meister, A., Sober, H. A., Peterson, E. S., *J. Biol. Chem.*, 1954, v206, 89.

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Antigenic Differences Between Two Strains of Respiratory Syncytial Virus. (28221)

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Respiratory syncytial virus (RS) is now recognized as one of the most important causes of severe respiratory tract illness in infants and young children(1-6). It was recently shown that hospital admissions for bronchiolitis or pneumonia in infants less than 6 months of age were 4 to 5 times greater during months of RS virus prevalence than during months when the agent was quiescent(6). There is, therefore, considerable interest in development of a vaccine against this agent. One important factor in preparing an effective vaccine is a thorough knowledge of the antigenic composition and extent of antigenic variation of the agent.

Early studies suggested that there was only one serotype of RS virus(1,7). However, we have reported that the A-1 strain of RS virus is not neutralized by hyperimmune guinea pig or rabbit serum prepared against the Long strain(8). Recently we have studied a strain of RS virus which differs even more from the Long strain. The nature and extent of this antigenic difference are reported here.

Materials and methods. Virus strains. The Long strain of RS virus was isolated in 1956 from a child with pneumonia(9). It was used in this study after 10 passages in human continuous cell lines (KB, Chang liver and HEp-2). The CH 18537 strain of RS virus was isolated during 1962 from a child with upper respiratory disease. This strain was