This did not occur until 24 hours of exposure, however, while the fasting mice in our studies remained at altitude about one minute.

Many changes associated with lipemia, reviewed by Ahrens(12), could contribute to deficient oxygen transport and would lend support to the possibility that fats are in themselves detrimental to tolerance to hypoxia. We must conclude from our data, however, that the apparently adverse effect of a high fat diet on survival of mice exposed to altitude is a result of carbohydrate deficiency rather than lipid excess.

The difference between the results of Mosinger and Kuhn and those presented here may be explained by inclusion in our series of a group of fasting animals and of groups fed sucrose. Although our data do indicate that a diet consisting of fat alone is associated with a significant decrease in survival time, they more clearly reveal the positive role played by carbohydrate.

Summary. Mice fed large amounts of carbohydrate prior to exposure to altitude hypoxia survive at least 5 times longer than fasting animals and significantly longer than animals fed their normal diet. When fed lipids of varying saturations while fasting, their tolerance is the same as that in the fasting state alone. Large amounts of fat added to the regular diet failed to decrease survival. These results demonstrate a salutary effect of carbohydrates on tolerance to hypoxia. They show as well the inability of lipids to alone promote survival. Possible mechanisms for the protection provided by carbohydrate are discussed.

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Amylase in Pancreas, Intestine, Liver, and Serum During Fasting, Non-protein Diet and Realimentation.* (24795)

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Apparently any meal is mixed with so much endogenous protein that in the small gut the resulting amino acid mixture tends to remain constant(1,2,3). This endogenous protein arises primarily from secreted enzymes and glycoproteins, as well as sloughed mucosa, and most of it may be hydrolyzed and recovered by absorption. The mass of mucosal cells entering lumen of alimentary tract in man may be as much as ½ lb/day(4). The alimentary tract obviously contains a fairly large and mobile protein reserve. This was demonstrated by changes in total N content of some abdominal viscera during fasting, non-protein feeding and realimenta-

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TABLE I. Amylase Activity* in Pancreas, Small Gut, Liver and Blood Serum.

Sample		Controls (8)†	Fasting 1 day (6)	Fasting 2 days (6)	Fasting 4 days (6)	Fasting 8 days (5)
Pancreas	Wt, g	.93	.81	.68	.63	.54
	Amylase	627 ± 98‡	496 ± 32	341 ± 60	248 ± 31	79 ± 15
Small gut	Wt, g	5.71	4.68	4.44	3.74	2.72
	Amylase	599 ± 36	584 ± 46	386 ± 56	375 ± 26	189 ± 9.3
Liver	Wt, g	10.15	7.35	6.50	6.01	3.09
	Amylase	191 ± 18	144 ± 8.8	118 ± 5.1	90 ± 5.6	37 ± 5.8
Serum	Amylase	301 ± 20	229 ± 12	213 ± 13	164 ± 10	84 ± 23

* Smith and Roe units in whole pancreas, small gut and liver, and in 100 ml of serum.
† No. of animals in parentheses.
‡ Multiply pancreas values by 100.

tion. Stomach lost only 8% of its N, but small gut, pancreas and liver lost approximately 50% of their N in 8 days of fasting. Feeding non-protein diet induced stomach and pancreas to lose more and the small gut to lose less N than in fasting(5). More specific information was sought than afforded by determinations of total N. Experiments described below were concerned with determination of amylase in pancreas, small gut, liver and blood serum in fasting, feeding non-protein diet, and realimentation. Amylase doubtless represents a small fraction of total protein turnover in these tissues but it is significant that changes in amylase activity generally parallel changes in total N content previously reported(5).

Methods. Male albino rats (240-300 g) of Wistar strain were caged individually at 26-28°C and fed complete commercial ration for 1 week before the experiment. Control animals were taken directly from feed and sacrificed for determinations of amylase activity. Fasting animals always had access to water. The diet used in realimentation contained the following: egg albumin, 12.8%; sucrose, 56.9%; cottonseed oil, 17.4%; salts (USP XIII), 4.5%; vitamin mixture, 2.6%; and cellulose flour, 5.8%. The nonprotein diet was made by substituting an equal quantity of sucrose for egg albumin. The energy allowance was 121 Cals/day/kg^{¾}, sufficient for maintenance of original body weight. Food was always available and was more than 95% consumed. Under light ether anesthesia, blood was obtained by cardiac puncture and allowed to clot at 1-4°C. The thoracic aorta and vena cava were divided to permit most of remaining blood to pool above the diaphragm. Pancreas, small bowel and liver were quickly removed, taking care to exclude mesentery and adipose tissue. The gut was slit and its contents washed away gently in 2 changes of ice cold Ringer solution. Excess moisture was removed on paper towel. After weighing, entire organs were ground with chilled sand in chilled mortar for 3 minutes. Samples were diluted with chilled solution (phosphate buffer, pH 7.2, 1 part and 4 parts of Ringer solution) as follows: pancreas 1:1000, (w/v); liver and intestine 1:10, (w/v); blood serum 1:10, (v/v). Samples were well mixed, centrifuged and the supernatant used for determination of amylase activity. A 3% solution of soluble starch was used for pancreas and intestine instead of 1.2% solution specified in the original method for serum and urine(6). To avoid exceeding the range of the method preliminary estimates of amylase activity were made in each series.

Results. The results obtained from control and fasting animals are summarized in Table I. The "standard rat" was taken to be 275 g, corresponding to a metabolic body size of 0.380 kg^{¾}. Weights of the 3 viscera and their corresponding total amylase activities were computed on this basis. In controls, the whole pancreas contained about 100 times as much amylase as the whole small gut and 300 times as much as the liver. Total amylase activity was reduced to 13, 32, 14 and 28% of controls respectively in pancreas, small gut, liver and serum in 8 days of fasting. Departures from control values induced by fast-

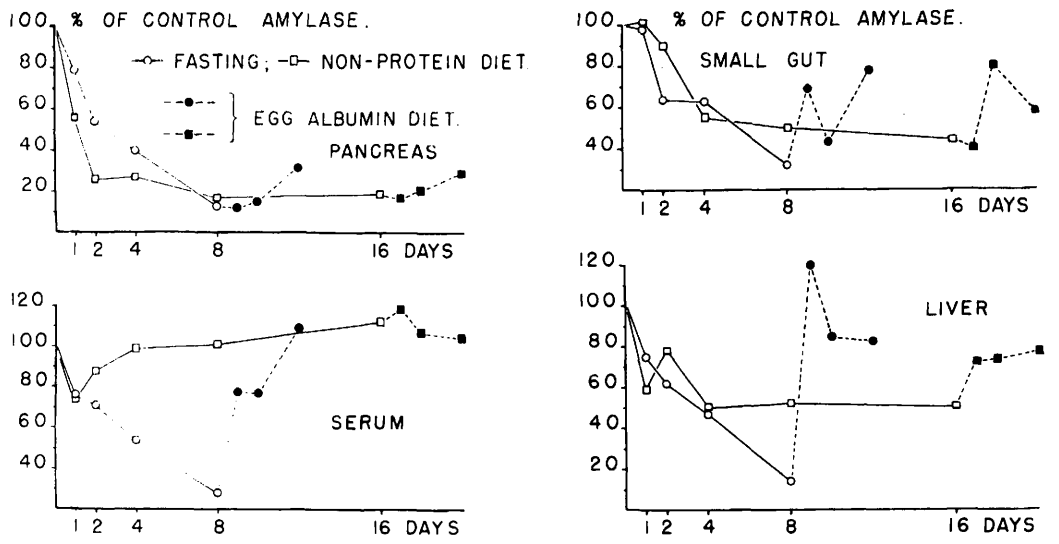

FIG. 1.

ing or ingesting non-protein diet, and effects of realimentation are shown in Fig. 1. All points on curves were derived from 6 rats and represent mean percentile changes from controls taken as 100%. Variability among animals represented in Fig. 1 was about the same as that indicated in Table I.

Discussion. In fasting, visceral and serum amylase activity declined steadily but in the small intestine it consistently declined the least. When non-protein diet was ingested, changes in amylase content were somewhat different from those seen in fasting. Feeding non-protein diet seemed to spare amylase activity early in small gut and later in serum (Fig. 1). Liver data on non-protein diet seem more erratic but the rise in amylase on second day is significant ($p<0.05$) and may represent a contribution from the gut which just began to decline on second day. On non-protein diet the pancreas was depleted of amylase activity much more rapidly than in fasting. At face value this is the opposite of a protein-sparing effect.

Any meal stimulates the pancreas to secrete a portion of its enzyme content. If amino acids are unavailable for resynthesis of enzyme proteins depletion becomes progressive. Absence of food stimulation probably accounts for the greater pancreatic amylase activity in fasting. The small gut is also a glandular viscus and secretes succus entericus in response to chyme delivered from stomach. The essential difference is that the intestinal mucosa is also the absorbing organ. As such it is ideally situated to provide for its own deficit of amino acids by removing them directly from the lumen. At present it is impossible to differentiate sharply between secretory and absorptive cells in the intestinal mucosa. Amino acids not retained by the gut are delivered to the portal vein and are available, next to the liver, and finally to the pancreas and other organs served by the systemic circulation. Previous work demonstrated that variations in total N, and hence probably in total protein, in these organs paralleled the changes in amylase activity reported here(5).

The mechanism for maintenance of normal serum amylase activity for most of the time during ingestion of non-protein diet is not apparent. Amylase is assumed present in blood serum because it escapes from cells which produce it either by diffusion or destruction of cell membranes. Equilibrium in serum between inflow and outflow of amylase is represented by normal control values. When non-protein diet was fed, the order and extent of the decline in amylase activity differed in viscera and serum, as noted above, but after the fourth day values for serum remained essentially unchanged. The large drop in amy-

lase in pancreas, gut and liver in the first 4 days may represent loss of an insoluble storage form of the enzyme presumed present in secretion granules. Concentration of soluble enzyme, as represented by newly synthesized enzyme just prior to molecular aggregation and storage in granules, may remain essentially unaltered under these conditions. This might account for reestablishment of diffusion equilibrium with serum as suggested by the data.

Realimentation with a complete diet after 8 days of fasting evoked an immediate rise in amylase activity in gut and liver, followed the next day by a highly significant fall in both ($p<0.01$). Realimentation had no effect on pancreas for at least 2 days and very little in 4 days. Under the stimulus of feeding, secretion of pancreatic amylase apparently kept pace with its synthesis for several days. After 16 days on non-protein diet, realimentation with complete diet was accompanied by appreciable recovery of amylase activity in gut and liver. Serum failed to depart from normal and again pancreas made very little recovery in 4 days. The gut response seems more erratic than that of other viscera. This may be associated with the presence of much muscular and other non-glandular tissue in this organ which might compete successfully for available amino acids.

Summary. Male rats were fasted for 8 days or fed non-protein diet for 16 days. Amylase activity was determined at intervals in pancreas, small gut, liver and serum. In each instance fasting caused sharp and parallel drops in amylase activity. Feeding non-protein diet 2-4 days seemed to spare amylase in small gut and serum. Liver fluctuated in the first 4 days and then remained at half normal activity for 16 days. Pancreatic amylase activity was depleted more on non-protein diet than in fasting. Feeding induces increased secretion of pancreatic amylase which, with other endogenous proteins, is probably hydrolyzed in the intestinal lumen and its amino acids absorbed. The data suggest that intestinal mucosa by its absorptive function is able to remove amino acids for protein synthesis directly from gut contents, and the liver has next choice through the portal system. Finally pancreas and other tissues are supplied by way of the peripheral circulation.

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Incorporation of DL-Lysine-2-C^{14} into Melanin Pigment of Turkey Poult Feathers. (24796)

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Amino acid lysine is essential for normal feather pigmentation of turkey poults(1). Pigment in feathers is melanin in nature. It is desired to know whether lysine is a precursor for melanin synthesis in the intact poult by investigating incorporation of lysine-2-C^{14} into melanin granules of poult feathers.

Methods. Broad Breasted Bronze turkey poults of indeterminate sex, 3 to 4-day-old, were used and fed commercial poult starters. *DL-Lysine-2-C^{14} monohydrochloride.* Lysine with a specific activity of 0.3 mc/mM was obtained from Tracerlab Inc., Boston, Mass. A known weight of this sample was diluted with inactive DL-lysine hydrochloride and dissolved in water to give various solutions