was given a single injection of 2.5 mg/K. (Table III).

Summary. An acute phase protein (Cx-protein) has been produced in the rabbit by the administration of 3 cytotoxic agents: X-radiation, nitrogen mustard, and amethopterine.

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Effect of Partial Deficiency of Threonine on Enzyme Activity and Fat Deposition in Liver.* (20665)

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The activities of certain liver enzymes are affected by the quality and quantity of the protein and by the amounts of certain amino acids in the diet(1,2). The activity of liver xanthine oxidase, for example, drops when the level of either tryptophan or methionine in the diet is reduced(3,4). Liver fat deposition in animals fed diets *containing choline* is also affected by the quality and quantity of the dietary protein(5,6) and by the levels of individual amino acids. The accumulation of fat found in the livers of rats fed 9% casein diets is reduced when such diets are supplemented with threonine(5,7). Since partial deficiencies of amino acids affect both liver enzyme activity and liver fat deposition a study of the effect of a partial deficiency of threonine on the activities of certain liver enzymes has been undertaken in an effort to demonstrate a correlation between enzyme activity and fat deposition.

The enzyme systems chosen for the initial study were: xanthine oxidase, which is known to be sensitive to alterations in dietary protein; tyrosine oxidase, which is a soluble cytoplasmic enzyme system(8) yielding acetoacetate as an end product of tyrosine oxidation; succinic oxidase, which is a mitochondrial enzyme essential for the operation of the tricarboxylic acid cycle; choline oxidase which is a mitochondrial enzyme not involved in carbohydrate metabolism; and endogenous oxidation and decarboxylation, which give a measure of the oxidative metabolism of endogenous substrates.

Methods. Male weanling rats of the Sprague-Dawley strain weighing from 40 to 50 g were separated into similar groups of 12 animals each and were maintained in individual cages with raised screen bottoms. The animals were fed the experimental diets *ad libitum* and each animal was weighed weekly during the experimental period of 2 weeks. The basal ration was composed of the following: Sucrose, 81.5; casein, 9.0; DL-methionine, 0.3; DL-tryptophan, 0.1; corn oil, 5.0; salts IV(9), 4; and choline chloride, 0.13%. Vitamins were added to provide, in mg per 100 g of ration, thiamine, 0.5; riboflavin, 0.5; niacin, 1.0; calcium pantothenate, 2.0; pyridoxine, 0.25; biotin, 0.01; folic acid, 0.02; vitamin B_{12}, 0.002; and inositol, 10.0. Two drops of halibut liver oil fortified with vitamins E and K(6) were administered orally each week. DL-threonine, when supplemented in the ration, was added at a level of 0.36% and replaced an equal weight of sucrose. After 2 weeks 6 animals from each group were sacri-

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ficed for the determination of liver fat. The animals were stunned and decapitated and the livers were excised. Fat was determined by ether extraction of the dried and ground liver (10). The remaining 6 animals were used for the enzyme determinations. The animals were killed as described above. Each liver, immediately after being removed, was chilled in ice and blotted free of moisture. A weighed portion of each liver was homogenized with 5 volumes of ice-cold 0.039 M sodium potassium phosphate buffer (pH 7.3). Xanthine oxidase activity of the homogenates was determined by the method of Axelrod and Elvehjem(11) and choline oxidase by the method of Williams, Litwack and Elvehjem(12). Endogenous oxidation was determined by measuring oxygen uptake of the homogenates in the absence of substrate. Endogenous carbon dioxide evolution was determined by the "direct method" (13). For the determination of succinic oxidase 0.2 ml of a 0.4 M solution of sodium succinate was placed in the side-arm of a Warburg vessel and in the main compartment were placed 0.6 ml of 0.039 M sodium potassium phosphate buffer, pH 7.3, and 1 ml of the homogenate. The total volume was made up to 2 ml by the addition of distilled water to the side-arm. A control vessel containing distilled water instead of succinate was run simultaneously to obtain a correction for endogenous oxidation. For the tyrosine oxidase determination 0.2 ml of a 0.15 M suspension of L-tyrosine and 0.2 ml of 0.15 M sodium α-ketoglutarate were placed in the side-arm, and 1.6 ml of Krebs-Ringer phosphate buffer (pH 7.3) (13), and 1 ml of homogenate were placed in the main compartment. A control vessel containing distilled water in place of L-tyrosine was included. Potassium hydroxide, 0.2 ml of a 10% solution, was placed in the center well of each vessel, except those for the estimation of endogenous carbon dioxide evolution. The *homogenates* were kept in ice after preparation and were pipetted as soon as possible (about 5 minutes after preparation) into chilled, prepared Warburg vessels. The vessels were incubated at 37°C for 10 minutes to reach temperature equilibrium. The substrates or water were added from the sidearms and the stop-cocks were closed. Readings were taken at 10 minute intervals (30 minute intervals for xanthine oxidase) until the activities of the enzymes had reached a maximum and begun to level off. Endogenous respiration measurements (oxygen uptake and carbon dioxide evolution) were contained in all cases for 1 hour. *Xanthine oxidase activity* was calculated according to the method of Axelrod and Elvehjem(11). Succinic oxidase and choline oxidase were calculated from the series of 10-minute readings most nearly approaching a straight line when plotted against time. Tyrosine oxidase, endogenous oxygen uptake and carbon dioxide evolution, which appeared to be particularly unstable systems, usually reached a maximum during the first 10 minute interval and decreased in activity thereafter. Since it was considered that a better index of the activities of these systems would be obtained by taking into account both the actual activity and the stability of the enzyme system, the activity of the tyrosine oxidase system was calculated by averaging the first three 10-minute readings. The activities of the other two systems, endogenous oxygen uptake and carbon dioxide evolution, were calculated by averaging the six 10-minute readings obtained during 1 hour.

The *nitrogen content* of the homogenates was determined by a micro-Kjeldahl method.

The experiment, as outlined above, was carried out once and then duplicated in its entirety. A total of 48 rats was used in these studies.

Results. Results of the growth studies and liver fat determinations are presented in Table I. In both experiments the inclusion of threonine in the basal diet caused an increase in the rate of growth that was accompanied by a substantial decrease in the percentage of fat in the liver. The variation in absolute values from experiment to experiment can probably be accounted for by variations in the nitrogen content of different lots of casein.

Results of the enzyme determinations are presented in Table II. All values have been calculated on the basis of fresh weight of liver since the relative values remain unchanged when they are converted to a liver nitrogen basis (see footnote in Table II). The activities of the cytoplasmic or soluble liver enzy-

TABLE I. Effect of Additional Dietary Threonine on Growth and Liver Fat of Rats Receiving a 9% Casein Ration Supplemented with Methionine and Tryptophan.

Exp. No.	Growth			Liver fat		
	No. of rats	Basal	+ threonine	No. of rats	Basal	+ threonine
		g/wk			%	
I	12	13.7 ± 1.9*	19.5 ± 2.2	6	40.4 ± 2.1	20.7 ± 1.6
II	12	19.7 ± 1.8	25.3 ± 2.2	6	26.9 ± 2.0	12.9 ± 1.1

* Stand. error of mean.

TABLE II. Effect of Additional Dietary Threonine on Liver Enzyme Activity of Rats Receiving a 9% Casein Ration Supplemented with Methionine and Tryptophan.

Enzyme measured*	Exp. I		Exp. II		Total No. of rats	Avg of 2 exp.	
	Basal	+ threonine	Basal	+ threonine		Basal	+ threonine
Xanthine oxidase	10	64	25	50	24	17 ± 6†	57 ± 7†
Tyrosine "	83	307	86	338	20	84 ± 22	322 ± 19
Succinic "	2660	1740	2400	1520	22	2520 ± 200	1620 ± 160
Choline "	1620	1240	1560	960	20	1580 ± 60	1090 ± 122
Endogenous oxidation	1170	2040	855	1695	22	955 ± 190	1870 ± 110
Endogenous decarboxylation	630	1590	473	1155	21	560 ± 90	1370 ± 120

* Values expressed as $\mu l O_2$ or CO_2/g fresh liver/hr. No significant change in relative activity of any of the groups was observed when calculated on the basis of liver nitrogen.

† Stand. error of mean.

mes, xanthine oxidase and tyrosine oxidase, were lower in homogenates from animals receiving the basal diet without additional threonine. The reverse was true, however, of the mitochondrial enzymes, succinic oxidase and choline oxidase. Endogenous oxidation and carbon dioxide production, like the cytoplasmic enzymes, were considerably lower in the livers of animals which received no supplementary threonine.

Discussion. The reduced deposition of fat found in the livers of animals which received the basal diet supplemented with threonine is in agreement with the results of previous reports(5,7). Although the inclusion of threonine in the basal diet caused increased growth, little correlation has been observed between its action in reducing liver fat deposition and its growth-promoting activity(5,6).

Increased activities of the mitochondrial enzymes in the homogenates from animals receiving the basal diet were not anticipated. Since the capacity for oxidation of both succinate and choline is greater and since liver fat deposition is greater when threonine is limiting, these observations do not support the hypothesis that the higher liver fat of animals receiving the basal diet is the result of decreased synthesis of mitochondrial enzymes (7).

The differences observed in xanthine oxidase activity can be correlated directly with the level of threonine in the diet. Since liver xanthine oxidase is known to be sensitive to specific amino acid deficiencies such as tryptophan(3) and methionine(4), and since threonine is the limiting amino acid in the basal diet, these results indicate that the level of dietary threonine is also important in maintaining the activity of this enzyme. The reduced activity of tyrosine oxidase, which is also a soluble enzyme located in the cytoplasm, can probably be similarly explained.

The markedly lower endogenous respiration of liver homogenates from animals receiving the basal diet is indicative of a metabolic difference between the 2 groups. However, since differences in these endogenous measurements may be caused by differences in any one of a number of factors (*e.g.* supply of respiratory substrates, concentration of co-factors, activities of oxidative enzymes) it is not possible to link these changes directly with alterations in liver fat deposition. The possibility that the reduced endogenous respiration may be directly related to the accumulation of liver fat

is worthy of further investigation.

It is evident from the experiments reported in this paper that a partial deficiency of threonine causes marked changes in the activities of several liver enzymes as well as in the extent of liver fat deposition. Since these marked changes occur concurrently, it is suggested that the liver fat accumulation observed in animals fed 9% casein-sucrose diets is a reflection of an alteration in the activity of one or more enzymes important for normal liver function.

Summary. The effect of a partial deficiency of threonine in the rat on the activities of several liver enzymes and on liver fat deposition has been determined. Liver fat deposition, in confirmation of earlier reports, was higher in animals receiving the threonine-deficient diet. The activities of the mitochondrial enzymes, succinic oxidase and choline oxidase, were higher in liver homogenates from threonine-deficient animals. Endogenous respiration and the activities of the cytoplasmic enzymes, xanthine oxidase and tyrosine oxidase, were lower in the livers of animals fed diets deficient in threonine.

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Role of Bile Salts in Activity of Cholesterol Esterase. (20666)

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Nedswedski(1-3) showed that bile salts were required for the synthesis of cholesterol ester from colloidal solutions of cholesterol and higher fatty acids by pancreatic cholesterol esterase. In the absence of bile salts, the enzyme was completely inactivated in 24 hours, but if bile salts were present, it retained its activity almost completely for 10 days. It was postulated that the bile salts exert their effect by stabilizing the enzyme. Klein(4) observed that hydrolytic cholesterol esterase of cattle pancreas was activated by bile acids, especially cholic acid. Nomura(5), Sym(6), and Vercellone(7) reported similar observations. Recent reports(8,9) have presented improved procedures for studying hydrolytic and esterifying cholesterol esterase systems. Both systems were demonstrated in pancreas(8,9) dog serum(10), and rat intestinal mucosa(11). The enzyme or enzymes occurring in these three tissues were alike in respect to optimum pH, inactivation temperature, and in requiring bile salts for activity. Since the substrate emulsions employed in our experiments(8-12) were stable and uniform in composition in absence of bile salts, it