Production of Cx-Protein in Rabbits by Cytotoxic Agents. (20664)

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In 1951, Anderson and McCarty confirmed the occurrence in the rabbit of an acute-phase protein analogous to human C-reactive protein(1). They caused this material to be produced in the rabbit in response to the injection of either virulent pneumococci, virulent streptococci or of a vaccine containing killed typhoid organisms. Twelve to 24 hours after the injection of these agents the acute-phase protein could be detected in the sera of the animals.

In the several experiments reported here our aim was to attempt to produce this acute-phase protein (Cx-protein) in rabbits by the use of agents which do not produce an inflammatory response, but which have a direct action on the leukocytes and cells of the reticulo-endothelial system(2). For this purpose X-radiation, nitrogen mustard and amethopterine‡ were used. The rabbit sera containing Cx-protein and a specific antiserum prepared against this substance were drawn into capillary tubes following the method described by Swift, Wilson and Lancefield(3). The quantity of precipitate which resulted from mixture of antigen and antibody was expressed semi-quantitatively as varying from 0 to +++. The specific Cx-protein antiserum was kindly furnished to us by Dr. Harrison Wood of the Rockefeller Institute for Medical Research. The rabbits were bled at the beginning of the experiment and usually daily thereafter. The blood, which was obtained from the ear veins, was allowed to clot overnight in vaseline-lined tubes at room temperature; the serum was separated off the next day. Leukocyte counts were performed daily, but are not recorded here because there was no obvious correlation between the variation in the leukocyte differential counts and Cx-protein production. In these experiments the following results were obtained.

TABLE I. Appearance of C_x-Protein Following X-Radiation.

Rabbit	Hours after treatment 0	24	48	72	96	144	192
R 2357	—	+	—	—	—	—	—
2358	—	+	—	—	—	—	—
2359	—	++	+	+++	+++	+++	Dead

TABLE II. Appearance of C_x-Protein Following Nitrogen Mustard.

Rabbit	Hours after treatment 0	24	48	72	96	120	192
R 2360	—	++	+	—	++	++	++
2361	—	+++	++	—	—	+++	—
2362	—	+	—	—	—	++	—

TABLE III. Appearance of C_x-Protein Following Amethopterine.

Rabbit	Hours after treatment 0	24	48	72	96	120	144	168
R 2360	—	++	—	—	—	—	—	—
2361	—	++	—	—	—	—	—	—
2362	—	++	—	—	—	—	—	—

1. *X-radiation.* Three rabbits were irradiated for 30 minutes with a surface dose of 300 r.§ In 2 rabbits Cx-protein was produced in only small amounts; in the third, which produced more Cx-protein, death occured on the sixth day, and the rabbit was shown to have coccidiosis. (Table I)

2. *Nitrogen mustard.* Three rabbits were injected with nitrogen mustard (Boots); each received a single intravenous injection of 2mg/K. (Table II). It is interesting that in all three rabbits Cx-protein disappeared on the third day and in each case reappeared later.

3. *Amethopterine.* Three rabbits were injected with amethopterine (Lederle); each

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§ The rabbits were kindly irradiated by Prof. Mitchell of the Radiation Laboratory, University of Cambridge.

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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