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Effect of a Necrogenic Yeast Diet on Viral Hepatitis (M.H.V.$_3$) in Mice.* (26649)

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Certain yeast diets have long been known to produce liver necrosis in rats(1). In mice DeWitt and Schwarz(2) have recently produced necrosis affecting the liver, heart and some other organs by using a diet in which dried Torula yeast was the sole source of protein. They also showed that these lesions can be prevented by supplementation with inorganic selenium or alpha tocopherol. In chicks a Torula yeast diet produces exudates in muscle, fat and skin which can be prevented by the same supplements(3). We have investigated the effect of the necrogenic diet on experimental hepatitis produced in mice by M.H.V.$_3$ virus(4). It had previously been shown that an increase in protein intake greatly reduced the mortality from this infection. This effect could not be reproduced by individual amino acids(5) or selenium.‡

Methods. Female weanling Swiss Webster mice were used. Repeated blood examinations had shown these mice to be free of Eperythrozoon coccoides, a protozoan which enhances the severity of the hepatitis. The principal constituents of the experimental diets are shown in Table I. The necrogenic diet (Diet I) was similar to that of DeWitt and Schwarz(2). Diets II and III were identical with Diet I except for supplementation with selenium and alpha tocopherol respectively. Diet IV was similar to Diet I except for substitution of casein for yeast. Since only about half the yeast consists of protein, the 15% of casein in Diet IV supplied approximately the same amount of protein as the 30% of yeast in Diet I.

Groups of mice aged 17 days were fed unrestricted quantities of the various diets. Unlimited fresh water was supplied. The animals were weighed twice weekly. After approximately 4 weeks a few deaths started to occur in Group I, the members of which were fed the necrogenic yeast diet. After 31 days half the animals of Group I and most of those on Diets II, III and IV were inoculated intraperitoneally with 0.1 ml of the M.H.V.$_3$ virus preparation(7). This consisted of a 10% suspension in Gey's balanced salt solution of mouse liver recently obtained from infected animals and stored at −30°C. Cages were inspected daily and dead mice removed. Some in each group were autopsied.

Results. In the first experiment mice fed the necrogenic diet (Diet I) were compared with animals receiving a diet identical apart from supplementation with alpha tocopherol (Diet II). Both groups were compared with

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TABLE I. Composition of Experimental Diets (% by Weight).

Diet	Torula yeast, %	Casein, %	Dextrin, %	Stripped lard, %	α-tocopherol, %	Sodium acid selenite, mg %	Salts and vitamins, %
I	30	—	60	5	—	—	to 100%
II	30	—	60	5	.05	—	" "
III	30	—	60	5	—	.04	" "
IV	—	15	75	5	.05	—	" "

Torula yeast was kindly supplied by Lake States Yeast and Chemical Division, St. Regis Paper Co., Rhinelander, Wis.; "vitamin-free" casein and white dextrin were obtained from Nutritional Biochemicals Corp.; stripped lard from Distillation Products Industries, Rochester, N. Y. Salts and vitamins included cod liver oil, 1%; salt mixture No. 2 USP, 2.5%; vitamin mixture(6), 1%; choline chloride, 0.5%.

TABLE II. Number of Mice Dying Each Day after Injection of M.H.V.$_3$ Virus.

	Day									
	1	2	3	4	5	6	7	8	Total deaths	Mortality, %
30 mice on Diet I	1	0	14	6	4	0	0	2	27	90
28 " " " II	0	0	4	4	3	0	2	0	13	46
32 " " " IV	0	0	3	5	3	2	0	1	14	44

mice fed a casein diet approximately identical in protein concentration (Diet IV). All the 3 diets were adequate for growth. The mean weight increase produced by Diet I, the necrogenic yeast diet, was 6.1 g, slightly less than the 7.1 g gained by animals in Group II the diet of which was supplemented with alpha tocopherol. Animals receiving the casein diet (Diet IV) gained 13.4 g, approximately twice as much as those fed the yeast diets (Diets I and II).

The number of mice dying each day after infection is shown in Table II. The mortality of mice fed necrogenic diet (Diet I) was approximately double that of animals given selenium supplemented yeast diet (Diet II). This difference was statistically highly significant ($X^2 = 12.4$; $p<0.001$). Substitution of casein for yeast (Diet IV) reduced mortality from experimental hepatitis to a similar extent. Among 27 uninjected control animals receiving necrogenic diet (Diet I) there were 2 deaths during the same period. No deaths occurred among the uninjected control animals on Diet II and IV.

In the second experiment Diet I was compared with Diet II and III. Mean weight increase produced by the necrogenic diet (7.0 g) was again rather less than the gain by animals on the diets supplemented with alpha tocopherol (9.6 g) and selenium (8.5 g).

Number of mice dying each day after infection is shown in Table III. Mortality of animals given Diet I was again significantly higher than that of animals fed Diet II. Selenium proved equally effective in reducing the mortality from M.H.V.$_3$ infection (Diet III). During the same period there was one death among 30 uninjected control mice receiving Diet I. No deaths occurred among the uninjected control animals fed Diets II and III.

Discussion. The principal finding in this study was that a Torula yeast diet(2) which produced dietary necrotic degeneration in mice also increased susceptibility of these animals to experimental viral hepatitis. Alpha tocopherol and selenium which are

TABLE III. Number of Mice Dying Each Day after Injection of M.H.V.$_3$ Virus.

	Day									
	1	2	3	4	5	6	7	8	Total deaths	Mortality, %
30 mice on Diet I	0	0	5	15	7	1	0	1	29	96
45 " " " II	0	0	2	12	13	5	1	0	33	73
45 " " " III	0	0	1	9	7	11	5	0	33	73

capable of preventing the dietary necrosis (2) were effective also in reducing mortality of mice fed the necrogenic diet and infected with M.H.V.$_3$ virus. In chicks a Torula yeast diet produces multiple exudates. Like necrotic degeneration in mice these lesions can be prevented by both alpha tocopherol and selenium(3). Our experiments have provided more evidence that very small amounts of selenium can often replace Vit. E as an essential nutrient. We have also shown that mortality of mice infected with experimental viral hepatitis and receiving a casein diet supplying 15% of protein is similar to that of animals receiving a yeast diet of similar concentration and supplemented with alpha tocopherol. Although casein and yeast had a similar effect on mortality, casein was considerably more effective in stimulating growth than yeast supplemented with alpha tocopherol. This result confirms previous observations suggesting that the growth-promoting and anti-infective effects of protein are independent of one another(5).

Summary. A necrogenic Torula yeast diet increased the susceptibility of mice to experimental viral hepatitis. Supplementation of this diet with alpha tocopherol or selenium reduced the mortality of this infection. The susceptibility of mice fed a casein diet resembled that of animals receiving a yeast diet of similar protein content and supplemented with alpha tocopherol.

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Precursors of Etiocholanolone and Androsterone in Adrenal Carcinoma. (26650)

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Most patients with adrenal carcinoma excrete increased amounts of etiocholanolone (E) and variably increased amounts of androsterone (A) resulting in an E/A ratio well above the normal range(1,2). This has been interpreted to mean that some of the E originates from C_{21}-steroids whose metabolism leads to 17-ketosteroids (17-KS) of the 5β-series predominantly. Since the relative amounts of 5α- and 5β-17-KS may be related not only to the precursors but to the metabolic transformations of any particular precursor, tritium-labeled dehydroepiandrosterone (DHA) has been used to study the origin of a urinary A and E in patients with adrenal carcinoma. The secretion rate of DHA was also estimated in these patients.

Materials and methods. Five mg of DHA acetate were added to one millicurie of 7-tritium-DHA acetate (New England Nuclear Corp.), the material chromatogrammed in phenyl Cellosolve/heptane (I), eluted, and hydrolyzed with 0.067 N NaOH. It was then successively chromatogrammed in ligroin/propylene glycol (II), and isooctane/methanol:water, 10/8:2 (III). The DHA then had a specific activity (S.A.) of 51 $\times$ 10^6 cpm/mg.

The samples were counted in the Packard Tri-Carb liquid scintillation spectrometer with an efficiency of approximately 15%. Sufficient counts were accumulated to give a standard error of no more than 5%.

Urine was collected for 48 hours after intravenous injection of 2.1 $\times$ 10^6 cpm of DHA. The urines were treated as previously