Production of Dietary Necrotic Liver Degeneration Using American Torula Yeast. (18935)

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Dietary necrotic liver degeneration† was first recognized as a deficiency disease when Weichselbaum maintained rats on a cystine free diet(1). It is entirely different from fatty liver and cirrhosis, as first pointed out by Daft, Sebrell, and Lillie(2).

Necrotic degeneration can be produced by a wide variety of rations (Table I). All of these diets seem to be deficient in vitamin E and low in sulfur containing amino acids, especially cystine. The deficiency can be induced by diets which contain pure amino acids in place of protein, as reported incidentally by DuVigneaud, Dyer and Kies in a paper dealing with transmethylation(3). The protective action of cystine, discovered by Weichselbaum, was independently confirmed by Daft, Sebrell, and Lillie(2), Hock and Fink(4,5), Glynn, Himsworth and Neuberger (6), and others.

It has been shown since that dietary necrotic liver degeneration in most instances develops only when cystine and vit. E are insufficiently supplied at the same time and that each of these substances alone is able to protect(7). The significance of vit. E as a protective agent against necrotic liver degeneration was discovered by Schwarz(8).‡ The finding was confirmed by Gyorgy(9) and by Himsworth and Lindan(10) and Goettsch(11). It is possible that at least one other not yet identified factor may be protective against the disease(12). The question of whether necrotic liver degeneration, induced by different diets, constitutes an indivisible entity or whether several kinds or types of necrotic degeneration have to be distinguished deserves further investigation.

The deficiency can be induced in growing rats when the protein requirement is met by large amounts of yeast in purified diets(4,5). However, only certain yeasts have this effect, whereas others do not. Most European yeasts examined produced the deficiency in almost 100% incidence(7) whereas most American yeasts did not induce any degeneration or produced the deficiency only in a small percentage of animals after extended experimental periods. The lesions have been found using

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† "Degeneration" is defined as progressive deterioration of tissue in which its vitality is diminished, whereas "necrosis" means death of a circumscribed portion of tissue. "Dietary necrotic liver degeneration" is applied in this paper to define a deficiency disease which in previous publications has been called "hemorrhage"(1), "hemorrhage and necrosis" (2,6, etc.) or "liver degeneration"(7,8). The defect is characterized by a degenerative change which eventually leads to acute extensive liver necrosis, hemorrhages and death.

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3. DuVigneaud, V., Dyer, H. M., and Kies, M. W., *J. Biol. Chem.*, 1939, v130, 325.

4. Hock, A., and Fink, H., *Z. physiol. Chem.*, 1943, v278, 136.

5. Hock, A., and Fink, H., *Z. physiol. Chem.*, 1943, v279, 187.

6. Glynn, L. E., Himsworth, H. P., and Neuberger, A., *Brit. J. Exp. Pathol.*, 1945, v26, 326.

7. Schwarz, K., *Z. physiol. Chem.*, 1948, v283, 187.

8. Schwarz, K., *Z. physiol. Chem.*, 1944, v281, 109.

9. Liver Injury, Transactions of the Sixth Conference, Josiah Macy, Jr., Foundation, New York 1947.

10. Himsworth, H. P., and Lindan, O., *Nature*, 1949, v163, 30.

11. Goettsch, M., *Fed. Proc.*, 1950, v9, 177.

‡ The journal was republished in 1945 in the United States under the auspices of U.S.O.A.P.C., by J. W. Edwards, Ann Arbor, Mich.

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TABLE I. Diets Producing Necrotic Liver Degeneration in Rats.

Cystine-free (corn starch 80%, dry milk powder 16%)	Weichselbaum(1)
Cystine-free (amino acids in crystalline form)	DuVigneaud, Dyer and Kies(3); Glynn, Himsworth and Neuberger(6)
Low casein (10% or less)	Daft, Sebrell and Lillie(2); Gyorgy and Goldblatt(19); Hove and Harris (16)
Casein VI (alkali-treated, 15%)	Schwarz(17)
Yeast	Hock and Fink(4,5); Glynn and Himsworth(18); Schwarz(7); Gyorgy and Goldblatt(20)
High cod liver oil	Schwarz(21)
Potato protein, pea protein, cereal protein, Gelatin (in various combinations)	Hock and Fink(22)
Soy bean meal (raw, fat extracted)	Matet, Matet and Fridenson(23)

18% or less of American bakers yeast(13,14). This amounts to about 7% of protein in the diet which is inadequate in various amino acids. The resulting damage is complicated by these defects(15).

The peculiar discrepancy between American and European yeasts in regard to necrotic liver degeneration is not due to differences in variety, since very different strains of yeast have the same property and apparently not due to technical differences during production. It appeared to us, however, that the reason for the dissimilarity might be found in differences of media applied for the cultivation of the yeasts, grain products, *i.e.* corn steep liquor being more important ingredients in the U.S.A., whereas beet molasses, sulphite liquor, and wood hydrolysate are more widely used in Europe. Following this line of thought we found that torula yeast, grown in the United States in a simple medium (sulphite liquor) can be used for the induction of liver degeneration as well as European samples.

Experimental. Weanling rats of the Sprague-Dawley strain (National Institutes of Health Colony), weighing usually 35 to 40 g were divided into groups of 10 animals of equal weight, sex, and litter distribution. The animals were housed in individual elevated wire mesh cages. Weights were recorded once or twice weekly. Diets and tap water

TABLE II. Composition of Diets.

	Control diet	Torula diet	Yeast K diet
Casein*	15	—	—
Yeast	—	30	30
Sucrose†	74	59	59
Salt‡	5	5	5
Lard, Vit E-free§	5	5	5
Vitamin powder‖	1	1	1

* "Vitamin free," alcohol extracted.

† Commercial, regular grade.

‡ NaCl 54 g, $Na_2HPO_4 \cdot 7H_2O$ 91.5 g; $CaHPO_4$ 145.0 g; KH_2PO_4 258 g; Ferric citrate ($FeC_6H_5O_7 \cdot 3H_2O$) 32.5 g. Ca lactate ($CaCO_3H_5O_3)_2 \cdot 5H_2O$) 349 g; $MgSO_4$ 70 g; KJ·715 g. After mixing, the following are added: $MnSO_4 \cdot 7H_2O$ 325 g; $ZnSO_4 \cdot 7H_2O$ .4959 g; $CuSO_4 \cdot 5H_2O$ .285 g.

§ Vitamin E-free animal fat, stripped by molecular distillation (Distillation Products Inc., Rochester, N. Y.).

‖ Lactose 97.861 g, thiamine HCl 40 mg, riboflavin 25 mg; pyridoxine HCl 20 mg; D-calcium pantothenate 44 mg; choline chloride 1 g; niacin 1 g; menadione (2-methyl-1, 4-naphthoquinone) 10 mg.

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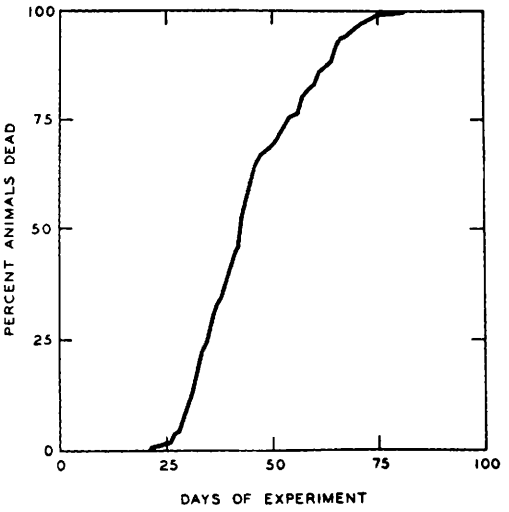

FIG. 1. Death from dietary liver degeneration. (Average of 15 experiments, Aug. 17, 1949-May 17, 1950.)

were given *ad libitum*. The diets (Table II) were prepared in dry form and fed in porcelain cups. A standard diet containing 15% purified casein was used for comparison. In yeast-diets, casein was replaced by 30% yeast, the difference of 15% was compensated for by a corresponding reduction in sucrose. The torula yeast was obtained from the Lake State Yeast Corp., Appleton, Wis.; Yeast K (dried, debittered brewer's yeast) was purchased from Anheuser Busch, Inc., St. Louis, Mo.§ The common water soluble vitamins and vit. K were incorporated routinely irrespective of the vitamin content of the yeast (Table II). Fifty γ of crystalline vit. A alcohol and 1 γ of crystalline vit. D_2 dissolved in 0.025 cc of ethyl laurate were given orally once weekly.

A thorough autopsy was performed on each animal. The effect of protective supplements was evaluated by comparing the average reciprocal survival time (multiplied by 100 in order to obtain suitable figures). This presents a mathematical expression for the velocity of the development of death. For example, for an animal surviving 25 days, the reciprocal is $\frac{1 \times 100}{25} = 4.0$. For animals surviving indefinitely the figure O is assigned. Average values for groups were calculated and compared.

Results. In animals on 15% alcohol extracted "vitamin free" casein necrotic liver degeneration has never been found either in these or in other extensive tests. When the torula diet was fed, the rats succumbed to necrotic liver degeneration in 100% incidence. Two groups of 10 animals on the yeast K diet grew well, and were sacrificed after surviving 70 and 145 days respectively. Their livers were normal.¶

Average results on torula diet. In 15 consecutive experiments with 14 groups of 10 and 1 of 12 animals on the torula ration, 145 out of 152 animals died with the necrotic liver disease; the other 7 rats died with complications (6 pneumonias, 1 kidney degeneration) during the test. The average survival time on the torula diet was 45.5 (22-83) days. The time mortality curve (Fig. 1) shows that death occurred, starting at the 22nd day, at a rather constant rate until about the 48th day. From there on the mortality rate declined.

Effect of L-*Cystine.* When L-cystine was added to the torula ration, increasing protection was afforded by increasing amounts (Table III). On a .1% L-cystine supplement all animals died, survival being slightly prolonged, as indicated by a 17.5% reduction of the average reciprocal survival time. Almost complete protection was provided when the ration was supplemented by .5% L-cystine from the start, or when .5 or 1.0% were added at the 26th day of the experiment.

Protective effect of vitamin E. The strong protective action of vit. E, formerly demonstrated in experiments with European yeast diets(7), is shown in Fig. 2. A supplement of 5 mg % alpha tocopherol acetate in

§ It is emphasized that the results reported in this publication do not allow any interpretation regarding the usefulness of various yeasts in animal nutrition since these materials were fed as the sole source of protein in highly purified, vit. E deficient rations.

¶ Two other American yeasts (yeast G, cultured, primary dried *Saccharomyces cerevisiae,* Anheuser-Busch, and an USP dried brewers yeast of unidentified origin) have been tested for their ability to produce necrotic liver degeneration using a system somewhat different to that described herein. They, like yeast K, failed to produce the defect.

TABLE III. Effect of L-Cystine.

% L-cystine supplement in diet	No. of animals	Dead due to liver degen.	Avg reciprocal survival time (×100) a. Controls‡	b. Experimental group	% reduction of reciprocal survival time§
.1	10	10	2.3	1.9	17.5
.2	9	6‖	2.3	.74	67.8
.5	9	3¶	2.08	.36	82.7
.5*	10	1¶	1.94	.25	87.1
.5†	7	1**	2.49	.26	89.4
1 †	9	1**	2.49	.19	92.5

* Pairfed to controls. † Supplementation started at 26.day. ‡ Littermates on torula diet with no cystine supplement. § (a-b) in % of a. ‖ Terminated 100th day. ¶ Terminated 112th day. ** Terminated 177th day.

the torula diet prevented the disease in all instances. With 1.25 or 2.5 mg % degeneration occurred only after very prolonged experimental periods. Surviving animals killed at the end of these tests had macroscopically normal livers.

Description of the disease. Rats on the torula diet stopped growing after 3-4 weeks, without displaying any other symptoms. After 22 to 83 days they suddenly lapsed into a fatal period which took but a few days or hours. At the beginning of this striking phase the animal remains quiet though trembling, and the body feels cool. Sometimes short attacks of hyperactivity can be seen. There is a slowing of reflexes and gradual loss of consciousness until a deep coma is reached which is accompanied by occasional convulsions. This stage persists for one to several hours, during which time the heartbeat and breathing fade; the transition from life to death occurs slowly and quietly.

Upon dissection, the most outstanding changes are seen in the liver (Fig. 3). A

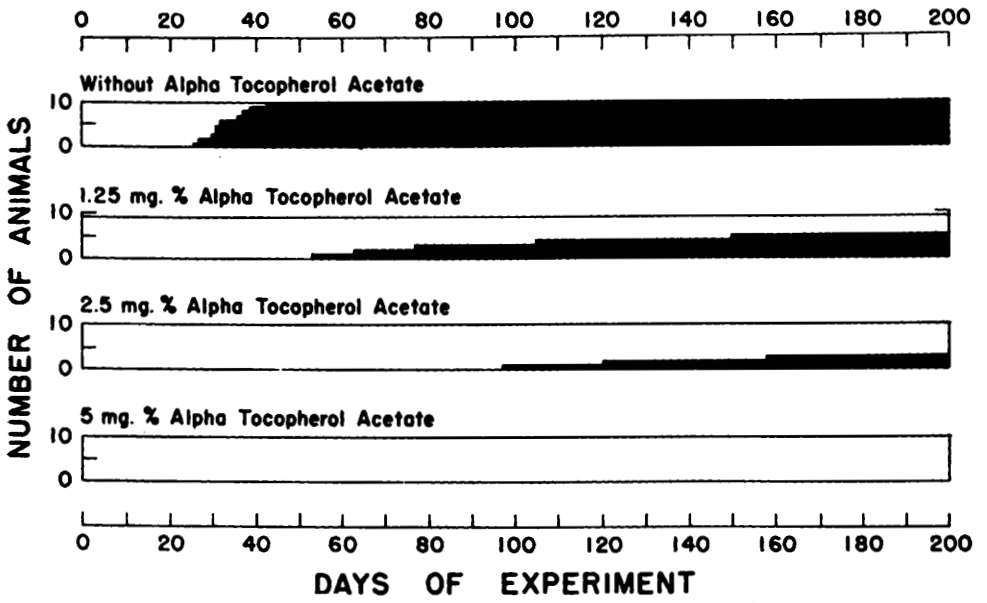

FIG. 2. Inhibition of liver degeneration by alpha-tocopherol acetate.

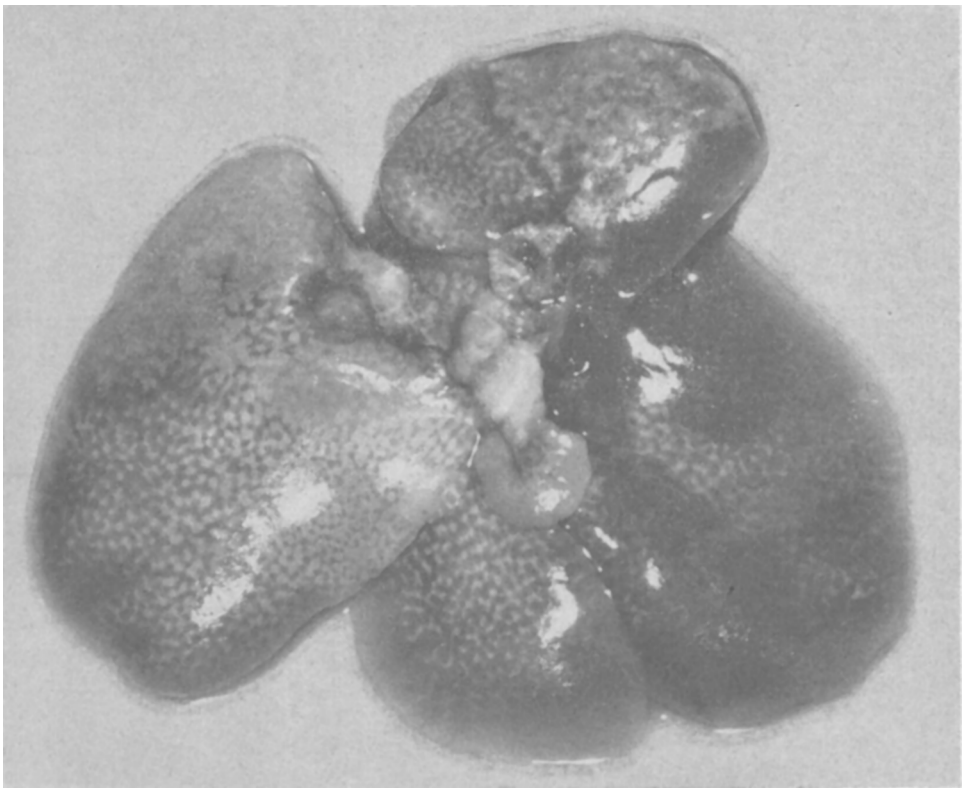

FIG. 3. Dietary necrotic degeneration of rat liver.

macroscopic description and histological analysis will be published separately.

The severe damages of the liver, which are precipitated in the short terminal period, are apparently the reason for the sudden death. In many of the animals degenerative changes can also be seen in the forestomach and the kidneys, and acute lesions are frequently found in the lung and the lymphatic system.

Ulcers of the forestomach of pinpoint to millimeter size (Fig. 4) occurred in about 51% of the rats. The lesion has been described previously(16), it is prevented by vit. E and seems to belong to the cycle of changes pertinent to dietary necrotic liver degeneration.

The kidneys of the rats which died displayed a whitish intercorticomedullary zone in about 87%. This white, often incrusted, area resembles that seen after the feeding of adenine, and might be caused by accumulation of dioxyadenine or similar products, due to the large amount of nucleic acids present in the yeast. Macroscopic defects of the cortex were rare.

Changes which are directly connected with the terminal phase of life occur in the lung. Acute lung edema was present in about 40% of animals, congestion and hemorrhages were seen in most of them. Hemorrhage also appeared in the gastrointestinal tract. The abdominal and mediastinal lymph nodes were hemorrhagic in many cases.

Summary. American torula yeast, when used as the sole protein source in Vit. E free diets, induced dietary necrotic liver degeneration in rats in the same manner as reported for European yeasts. Other American yeasts did not produce the defect. With 30% torula yeast in the diet the incidence of liver degeneration in 15 consecutive experiments was 100%, the average survival time was 45.5 days.

L-Cystine reduced the incidence of the defect considerably; however, even 1.0% did not give full protection when supplementation

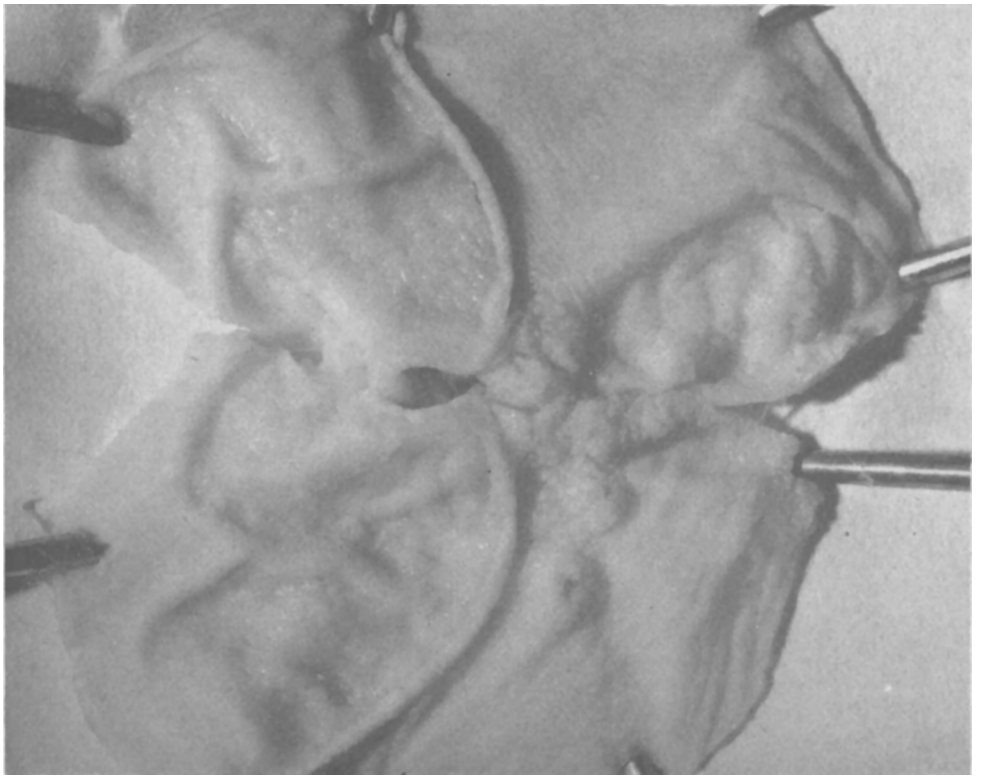

FIG. 4. Ulcers in forestomach of rat on torula diet.

started at the 25th day. Five mg % vit. E prevented the defect completely.

Necrosis of the liver developed very suddenly after an experimental period of 22 to 83 days and was accompanied by kidney lesions, forestomach ulcers, acute lung edema and hemorrhages in the lungs, in the lymphatic tissues and occasionally in the intestine.

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In Vitro Effect of Ethyl Alcohol on Respiration of Rat Liver and Kidney Slices.* (18936)

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In studies (unpublished) on the *in vitro* effect of testosterone on tissue respiration, ethyl alcohol was used as the solvent for the

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