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CARDIAC AND HEPATIC LESIONS IN MICE FED YEAST-PROTEIN DIETS
I. DIETS CONTAINING BRITISH BAKERS YEAST‡

INTRODUCTION

Massive necrosis of the liver occurs in rats restricted to diets in which yeast is the sole source of protein.[1-4] The present report considers the cardiac and hepatic lesions that developed in mice fed diets in which British yeast was the source of protein. In addition, studies have been made of the possible protective actions of dietary supplements (cystine or a-tocopherol) and of the substitution of casein for a portion of the yeast content of the diet. British bakers yeast was used because it has frequently served as the protein component of diets that produced a high incidence of hepatic necrosis in rats.[11, 14-17, 19]

MATERIALS AND METHODS

Animals: Mice of the AB stock (Strong A x Bagg albino) were restricted to the yeast diets when 10 weeks of age. From the time of weaning (30 days) these mice had been fed the stock colony diet of Purina Fox Chow. Males and females were used in approximately equal numbers.

Diets: The composition of the diets was as follows:

I. British yeast diet (BYD), 114 mice:

British bakers yeast§	180 gm.
Corn starch	666.5
Salt mixture (No. 2, U.S.P. XIII)	40
Peanut oil	100
Cod liver oil	4.5
Vitamin powder (see below)	10

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§ Dried Baking Yeast (United Yeast Co., Ltd., London, England).
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Vitamin powder:	
Thiamine	0.5 gm.
Riboflavin	0.25
Ca. pantothenate	1.0
Pyridoxine	0.2
Nicotinic acid	0.2
Powdered sugar	997.05

II. BYD plus vitamin E, 45 mice:

These mice fed the British yeast diet (BYD) also received vitamin E as an intramuscular injection of 2.5 mg. of a-tocopherol in saline solution twice weekly.*

III. BYD plus cystine, 25 mice:

Cystine (l-cystine) was added to the standard BYD diet at the level of 5 gm. per 1,000 gm. of diet.

IV. BYD plus casein, 44 mice:

Casein ("vitamin free") was substituted for one-half of the British yeast content of the standard diet. In this diet the yeast content was 9% and the casein content 9%.

Histological methods: Tissues were fixed in a modified[42] Lavdowsky's solution (alcohol, formalin, and acetic acid) except for the demonstration of lipids when 10% formaldehyde was used. Following use of the latter, frozen sections were cut and were stained with sudan IV or sudan black B. Methods for demonstrating fibrosis (Masson or Mallory), minerals (von Kossa), and PA-S- (periodic acid-Schiff's reagent) positive material were used as described by Lillie.[25] Basophilia and cytoplasmic ribonucleic acid of hepatic parenchyma were demonstrated by use of ribonuclease-hydrolysis and staining with basic dyes as described in an earlier study.[43]

RESULTS

Controls. All mice of this group were fed Purina Fox Chow. One hundred mice, 4 to 8 months of age, of the AB stock showed no cardiac, hepatic, or skeletal muscle lesions. In 46 mice of the same stock, 9 to 16 months of age, evidence of cardiac damage was limited to a small fibrotic area in one heart. Livers and muscle were normal. Diffuse pneumonia was a frequent "natural" cause of death in these mice.

Mice fed British yeast diets. The data concerning incidence of lesions are presented in Tables 1 and 2. The results discussed here are chiefly those related to the histopathological aspects of the lesions. The mice fed the diet in which one-half of the yeast was replaced by casein (BYD plus casein diet) were free of significant cardiac or hepatic lesions, but pneumonia was present in most of those that died. Cytoplasmic granulation, acidophilic and

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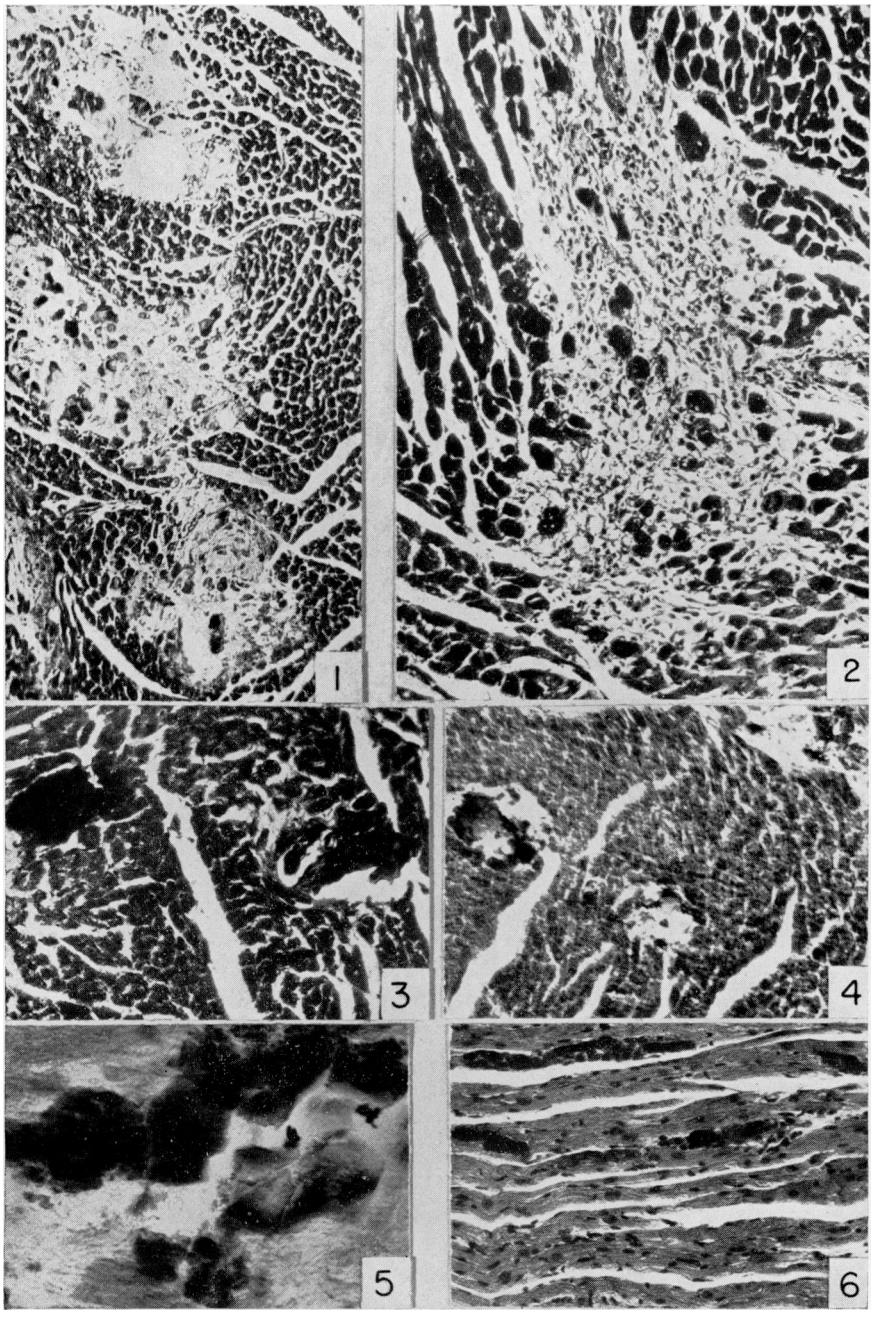

PLATE 1.

All figures (1-12) show hearts or livers of mice fed the nonsupplemented British yeast diet (BYD).

FIG. 1. Three areas of myocardial fibrosis. Masson, x100.

FIG. 2. An area of fibrosis as shown in Figure 1. The dark round structures are cardiac muscle fibers. These areas contain no deposits of mineral or ceroid. Masson, x150.

FIG. 3. Two deposits of mineral-ceroid in myocardium. von Kossa, x150.

FIG. 4. Same material as in Figure 3 except that the section was "demineralized" in nitric acid prior to application of the von Kossa technique. The intensity of the staining reaction is greatly reduced. x150.

FIG. 5. Mineral-ceroid material as shown in Figures 3 and 4. A routine paraffin-embedded section stained with sudan black B. This staining (plus acid-fast- and PAS-positivity) indicates that the material contains ceroid. x400.

FIG. 6. The myocardium is normal except for deposits of basophilic material. H. and E. x150.

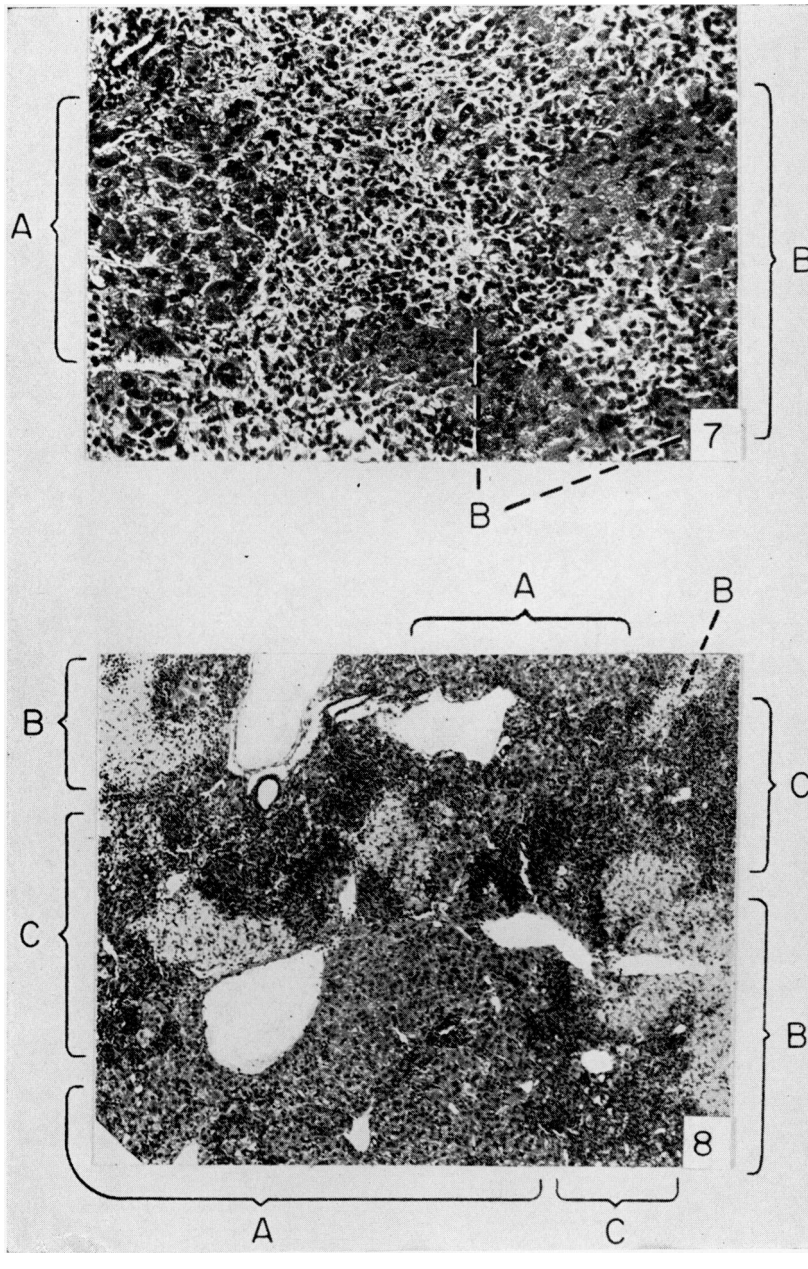

PLATE 2.

FIG. 7. Areas of relatively normal hepatic parenchyma (A) and of acute necrosis (B). The remainder of the section contains many small cells (macrophages, lymphocytes, and fibroblasts) which seem to be located in sites of earlier necrosis. H. and E., x150.

FIG. 8. Liver stained with gallocyanin-chromalum (at pH 2.5). Maximum cytoplasmic basophilia exists in areas of relatively intact parenchyma (A). Cytoplasmic basophilia is negligible in sites of active necrosis (B). Previous episodes of necrosis are indicated by the areas (C) containing many small cells (as in Fig. 7). x50.

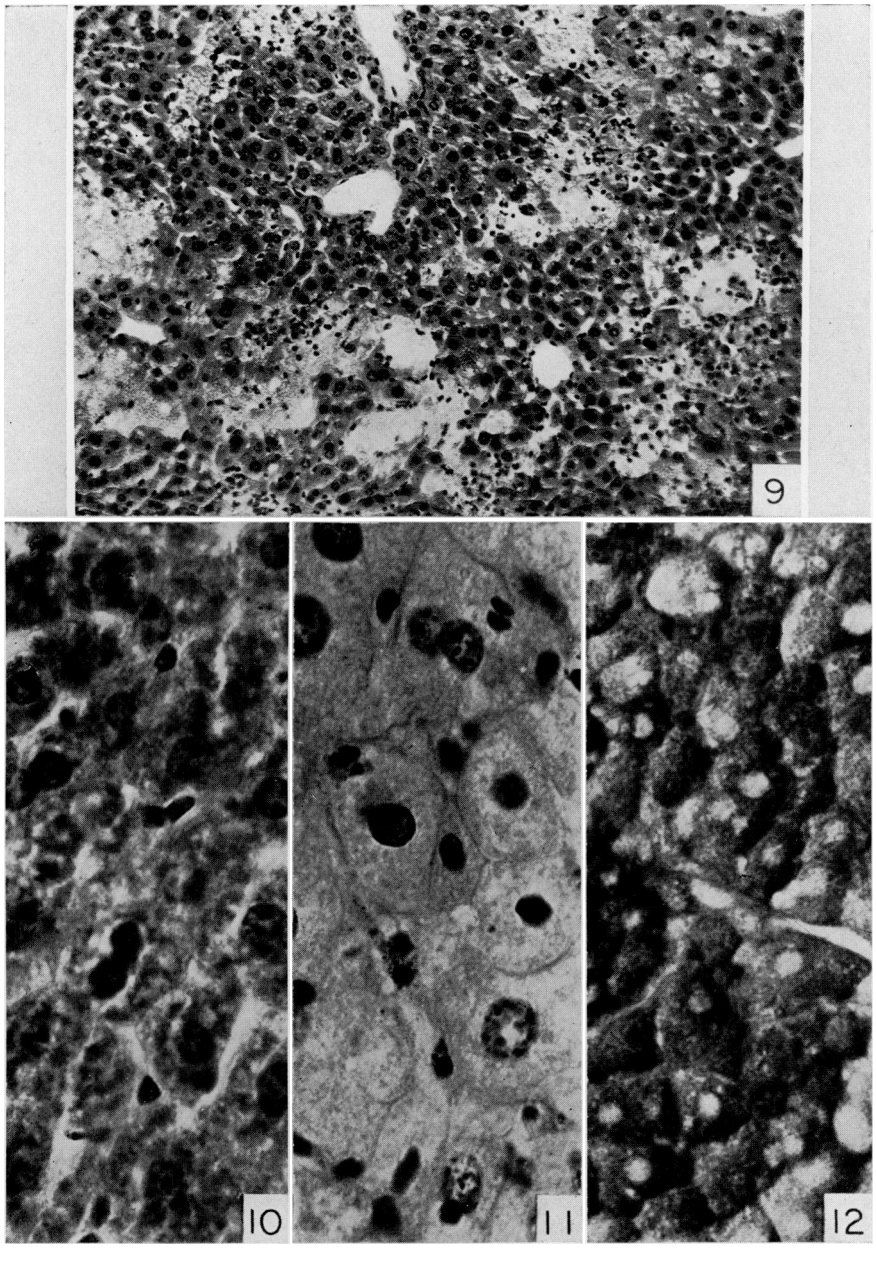

PLATE 3.

FIG. 9. Areas of intact hepatic parenchyma and of active necrosis (H. and E.).

FIG. 10. Normal liver stained with toluidine blue. The cytoplasm of the parenchymal cells shows the characteristic coarse, granular basophilia. x700.

FIG. 11. Area of "intact" parenchyma in liver showing necrosis. The cytoplasm is extremely hypobasophilic. Toluidine blue. x700.

FIG. 12. Same area as in Figure 11, but stained by the PA-S technique to show glycogen. The amount of PA-S-positivity approximates that of a normal liver. x700.

basophilic, of hepatic parenchyma was slightly reduced. Necrosis was not observed in hearts or livers.

In the other mice the histological characteristics of the lesions could not be correlated with the diet (BYD, BYD plus vitamin E, BYD plus cystine). The incidence of cardiac lesions was decreased in the vitamin E group and that of hepatic and myocardial necrosis increased in the cystine-supplemented group (see Tables 1 and 2).

TABLE 1. FINDINGS IN MICE FED THE BRITISH YEAST DIETS (BYD)

	The four BYD diets used:			
	BYD 114 mice	*BYD plus casein 44 mice*	*BYD plus vitamin E 45 mice*	*BYD plus cystine 25 mice*
No hepatic necrosis or myocardial lesions:	30%	100%	64%	20%
Days on diet:				
Average	55	228	49	22
Range	7–201	30–283	14–167	14–63
With myocardial lesions:	69%	0%	31%	76%
Days on diet:				
Average	97	.....	97	62
Range	30–283	.....	30–158	14–112
With hepatic necrosis:	8%	0%	11%	52%
Days on diet:				
Average	153	.....	124	50
Range	60–272	.....	109–134	7–112

NOTE: In a given dietary group the total per cent in excess of 100 is due to occurrence of both myocardial and hepatic lesions in some of the mice.

Mice fed the yeast diets (except the casein-supplemented group) weighed approximately 20% less than controls of the same age. Their coats were scanty and rough. These mice were generally inactive except upon manual or auditory stimulus when they were very hyperactive.

Hearts. The lesions were of two basic types, the incidence of each was approximately the same, and there was no evidence that one was the antecedent of the other. Obvious myocardial necrosis and fibrosis (see Figs. 1 and 2) represented one type. The other consisted of intramyocardial deposition of material that was basophilic and was also PA-S-, von Kossa-, and ceroid-positive (see Figs. 3-6). Fibrosis had occurred around these deposits. Although nitric acid was used to demineralize such areas it was

TABLE 2. INCIDENCE OF LESIONS (AS PER CENT OF TOTAL NO. OF MICE, KILLED OR DIED, DURING THE QUARTER); AND PER CENT OF TOTAL MORTALITY DURING QUARTER

	British yeast diets (BYD)			
	BYD 114 mice		*BYD plus vitamin E*	*BYD plus cystine*
	33 mice killed	*81 mice died*	*45 mice (all died)*	*25 mice (all died)*
First quarter (1-13 weeks)	*24 mice*	*58 mice*	*30 mice*	*22 mice*
No hepatic necrosis or myocardial lesions	29% (49*)	40% (44*)	83% (37*)	18% (28*)
Myocardial lesions	67% (69*)	59% (64*)	17% (39*)	73% (54*)
Hepatic necrosis	4% (64*)	3% (64*)	0%	55% (45*)
Mortality	.....	72% (56*)	67% (38*)	88% (46*)
Second quarter (14-26 weeks)	*4 mice*	*19 mice*	*15 mice*	*3 mice*
No hepatic necrosis or myocardial lesions	75% (112*)	0%	27% (123*)	0%
Myocardial lesions	25% (100*)	100% (115*)	60% (129*)	100% (103*)
Hepatic necrosis	0%	1% (110*)	33% (124*)	33% (112*)
Mortality	.....	23% (115*)	33% (126*)	12% (103*)
Third quarter (27-39 weeks)	*4 mice*	*4 mice*		
No hepatic necrosis or myocardial lesions	0%	25% (201*)	No	No
Myocardial lesions	100% (243*)	75% (233*)	Survivors	Survivors
Hepatic necrosis	25% (272*)	75% (233*)		
Mortality	.....	5% (225*)		
Fourth quarter (40-52 weeks)	*1 mouse*			
No hepatic necrosis or myocardial lesions	0%	No	No	No
Myocardial lesions	100% (283*)	Survivors	Survivors	Survivors
Hepatic necrosis	0%			
Mortality	.....			

* Average number of days on diet.

not possible to demonstrate myocardial necrosis within them. The surrounding fibrosis could indicate either replacement of necrotic myocardium or a reaction of the foreign body type to the mineral-ceriod deposits. Following exposure of the paraffin sections to 5% aqueous nitric acid, the reaction to the von Kossa technique was very faint (see Figs. 4 and 6), and the intensity of the PA-S reaction was diminished. The smallest of these deposits were basophilic (see Fig. 6) and PA-S-positive, but did not react to the von Kossa procedure. However, some mineral may have been removed by the acidic solution in which the tissues were fixed. The type of myocardial lesion did not seem to be related to the duration of restriction to the diet or to the presence or absence of vitamin E or cystine supplement.

The ventricles were the usual site of both types of lesions, otherwise there was a limited incidence of pericarditis and of diffuse myocarditis. Endocarditis and valvulitis were not observed.

Livers. The areas of necrosis usually involved several lobules (see Figs. 7-9). The parenchyma surrounding or adjacent to foci of necrosis appeared relatively intact in routine preparations stained with hematoxylin and eosin. Application of the ribonuclease hydrolysis-basophilic staining technique and of the gallocyanin-chromalum staining procedure[43] demonstrated a depletion of cytoplasmic ribonucleic acid in these parenchymal cells (see Figs. 8, 10, 11). Glycogen (PA-S technique) was not decreased significantly (see Fig. 12) and fat (sudanophilia of frozen sections) was not obviously increased in their cytoplasm. Neither cirrhosis nor ceroid was observed in the livers. In the livers of a very small number of mice (less than 1%) there were areas containing macrophages, lymphocytes, and a few neutrophils. These areas (see Figs. 7 and 8) were outside of the currently active sites of necrosis. Some of the cells may have been fibroblasts, but no fibres or ground substance could be demonstrated (see Fig. 7). Areas of similar appearance and composition in rats have been described as representing sites of previous episodes of dietary induced hepatic necrosis.[23] Mitoses were frequent in the areas of intact parenchyma, but the dividing cells were not adjacent to foci of necrosis. Often they seemed to be located in the centres of islands of fairly healthy parenchyma.

Skeletal muscle. It is obviously difficult to survey the voluntary musculature even in an animal as small as a mouse. Thigh muscles were studied routinely and in some instances, perivertebral muscles. In approximately one-half of the mice with cardiac lesions, the voluntary muscles contained mineral-ceroid identical with that described above as in the myocardium. Deposition of such material was not prevented by the vitamin E or cystine supplements.

Lungs. Diffuse pneumonia was observed in all animals that had cardiac or hepatic lesions. All animals that died showed pneumonia.

Reproductive tract. The seminiferous epithelium was generally atrophic except in the mice fed the diet in which casein had been substituted for one-half of the yeast. Spermatogenesis had not advanced beyond early spermatid forms. The type of tubular degeneration that occurs in vitamin E deficient rats[28] was not observed in the testes. Ovaries and uteri did not show deposition of ceroid that indicates vitamin E deficiency in the rat.[29, 30]

Other systems. The kidneys, adrenals, blood vessels, and peripheral nerves appeared normal except for renal abscesses in two mice. Adequate studies of the alimentary and central nervous systems were not made. Bones appeared normal in routine, decalcified sections stained with hematoxylin and eosin. Smooth muscle seemed normal. Oral and dental structures were not studied. The system of lymphatic organs showed no significant changes except a depletion in the number of mature lymphocytes which occurs in dietary restriction.

DISCUSSION

The results of this study demonstrate that in mice there exists a greater myocardial than hepatic susceptibility to the injurious effects of the yeast-containing diets. The histological pattern of necrosis in the mouse liver was similar to that observed in rats fed yeast-protein diet.[1-4, 14, 23] The frequency of hepatic necrosis in mice was approximately 10% of that observed in rats fed the same diet except in the cystine-supplemented group that attained more than one-half of the incidence in rats.[14, 17] The predominance of myocardial lesions in mice is of further importance because of the high incidence of such lesions in the same species[18] when fed a protein deficient diet.

Deficiencies of sulphur-containing amino acids (cystine and methionine)[8, 21, 22, 41] and of vitamin E favor the development of massive hepatic necrosis.[6, 24, 26, 40] Supplementation to eliminate these deficiencies decreases the incidence or prolongs the time of onset of hepatic injury.[16, 17, 24, 33-38] The relation of potential or real deficiencies of specific amino acids and of vitamin E in the induction of these lesions is further complicated by the marginal if not definitely inadequate amount of total protein (7%) in these diets.[18, 34-38]

Supplements of vitamin E decreased the total incidence of cardiac lesions without significantly altering that of hepatic necrosis (see Tables 1 and 2). Lesions of voluntary and of cardiac muscle occur in rats fed diets that are deficient in vitamin E.[9, 10, 13, 27, 30, 32] The synergistic or mutual effects of co-

existent protein and vitamin E deficiencies must be considered as causes of muscle lesions (cardiac or skeletal) and of hepatic necrosis.[7, 31] Prefeeding of rats with vitamin E and sulphur containing amino acids protects against subsequent production of hepatic lesions by feeding protein deficient diets.[26] Also, a low protein diet accelerates the injurious action of vitamin E deficiency upon the liver and voluntary muscles.[12]

The nutritional basis of the lesions observed here in mice fed the British yeast diet may be distinctly different from that responsible for the hepatic necrosis that occurs in rats fed the same diet.[14, 17, 28] The present data showing in mice a predominance of cardiac lesions and a relative infrequency of hepatic necrosis indicate some difference. Supplements of vitamin E or of cystine were completely effective in preventing hepatic necrosis in rats fed the British yeast diet for as long as 200 days.[17] In the mice vitamin E was only moderately "protective" for a limited time (see Table 2) and supplementation with cystine increased the necrogenicity of the diet in relation to both the heart and liver. Small supplements of cystine are known to exert "toxic" effects when concurrent deficiencies of lipotropic factors and amino acid sulphur exist.[5] However, the changes in the livers of the mice, particularly the lack of both significant intracytoplasmic liposis within the parenchyma and of cirrhosis, do not indicate a deficiency of lipotropic factors.

The feeding of casein-supplemented diets to the mice demonstrated that the presence of yeast in the ration was not in itself necrogenic to the liver and heart.

Hepatic necrosis has been produced in rats by feeding diets in which a considerable number of different yeasts served as the source of protein.[1-4, 34-38] The relevance of data furnished by such studies to the nutritional needs of mice is not known. The study of British yeast reported here and a preliminary survey[39] of two strains of American yeast* show that in mice the myocardium is highly susceptible to injury produced by deficiencies existing in diets in which yeast is the source of protein.

SUMMARY

When 70 days of age, 228 albino mice were restricted to a basic diet in which the sole source of protein was British bakers yeast. Of these, 45 mice received supplements of vitamin E (intramuscularly); 25 were fed the basic diet plus cystine (0.5 gm./100 gm. diet); and 44 were fed the

* Strains K2 and G of *Saccharomyces cerevisiae* yeasts furnished through the courtesy of the Central Research Department of Anheuser-Busch, Inc., St. Louis, Missouri.

basic diet except for substitution of "vitamin free" casein for one-half of the yeast content.

The remaining mice (114) were fed the basic diet without supplement of vitamin E, cystine, or casein. Within 97 days (average) 69% of these animals showed myocardial lesions. The incidence of hepatic necrosis was 8% at an average of 153 days.

Supplementation with vitamin E reduced the incidence of myocardial lesions without significantly altering the frequency of hepatic necrosis.

Addition of cystine increased both cardiac injury (76% incidence within 62 days) and hepatic necrosis (52% incidence within 50 days).

Mice of the casein-supplemented group were killed within 30 to 283 days. No significant lesions were observed.

The histological type of hepatic necrosis was similar to that observed in rats fed yeast diets. Myocardial lesions included necrosis, fibrosis, and deposition of ceroid and mineral (von Kossa) within damaged fibres. At least one-half of the mice with myocardial lesions also showed deposition of ceroid-mineral within skeletal muscle fibres. Livers did not show liposis, cirrhosis, or deposition of ceroid pigment.

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