THE EFFECT OF ALCOHOL ON THE CHOLINE REQUIREMENT*

II. Incidence of Renal Necrosis in Weanling Rats Following Short Term Ingestion of Alcohol

By GERALD KLATSKIN, M.D., and WILLARD A. KREHL, Ph.D.

(From the Department of Internal Medicine, and the Nutrition Laboratory, Department of Biochemistry, Yale University School of Medicine, New Haven)

(Received for publication, July 27, 1954)

In the accompanying paper (1) it is reported that rats maintained on a diet marginal in lipotropic activity developed fatty infiltration and fibrosis of the liver when given 15 per cent alcohol *in lieu* of drinking water or an isocaloric equivalent of sucrose over a period of 7 months. Since the hepatic changes could be prevented by supplementing the diet with choline or methionine, the possibility was considered that both alcohol and sucrose had induced a relative choline deficiency by increasing the number of calories consumed. However, it was found that restricting the caloric intake at the expense of sucrose in the basal diet did not abolish the effects of alcohol, although it did in the case of sucrose supplements. This suggested that (1) if alcohol increased the choline requirement it did not do so by augmenting the caloric intake, and (2) the modes of action of alcohol and sucrose were probably different, despite the similarity of the hepatic lesions they produced. It was pointed out that the effectiveness of lipotropic substances in preventing fatty infiltration and fibrosis of the liver did not conclusively establish the validity of the hypothesis that alcohol had created a relative choline deficiency. In particular the possibility was not excluded that alcohol had exerted a direct toxic action and that choline and methionine had merely hastened the removal of hepatic fat, as in the case of carbon tetrachloride poisoning (2).

The purpose of this report is to present confirmatory evidence indicating that alcohol *does* increase the choline requirement. To demonstrate this effect and to define it quantitatively, advantage was taken of the fact that choline deficiency produces highly characteristic renal lesions in the weanling rat (3–5).

Experiment 1

Methods.—Two hundred weanling Sprague-Dawley rats, weighing approximately 50 gm. each, were housed in individual cages in an air-conditioned room kept at 25°C. and a humidity of 50 to 55 per cent. The animals were divided into four groups of 50 each, as follows:—

A, basal diet and alcohol *ad libitum*.

* This work was carried out under the sponsorship of the Commission on Liver Diseases, Armed Forces Epidemiological Board, and was supported by the Office of the Surgeon General, Department of the Army.

B, pair-fed isocaloric controls (sucrose instead of alcohol).

C, pair-fed controls (no sucrose or alcohol supplement; therefore, not isocaloric).

D, basal diet *ad libitum*.

The low-choline basal diet employed was identical with that described by Strength, Schaefer, and Salmon (6) and had the following composition:

Peanut meal	30	per cent (50 per cent protein)
Casein (Labco vitamin-free)	6	" "
L-cystine	0.1	" "
Sucrose	39.5	" "
Lard	19	" "
Cod liver oil	1	" "
Salt mixture	4.4	" "
Vitamins:		*(mg. per kg. of diet)*
Thiamin hydrochloride	2	
Pyridoxine hydrochloride	2	
Riboflavin	4	
Calcium pantothenate	10	
Niacin	20	
i-Inositol	200	
Alpha-tocopherol	25	
Alpha-tocopherol acetate	25	
2-methyl-1,4-naphthaquinone	5	

Both the casein and peanut meal were purified by repeated extractions with hot 95 per cent methanol, as described by these authors. On direct analysis[1] the choline content was found to be 25.9 mg. per 100 gm. of peanut meal, or 7.77 mg. per 100 gm. of diet. It was estimated that the diet also contained 300 mg. of methionine per 100 gm. (6). Assuming that 3 moles of methionine are required for the biosynthesis of one of choline, the estimated total choline equivalent of the diet was 88.8 mg. per 100 gm. of diet.

The pair-feeding technic employed differed from that in our previous study (1) in that the consumption of food and alcohol in Group A was measured *daily* and the *average* for the group used in calculating the amount of basal diet plus sucrose and basal diet alone required for the controls in Groups B and C, respectively. As before, a 15 per cent aqueous solution of 95 per cent ethyl alcohol served as the only source of drinking water in Group A, and was dispensed in Richter tubes (7). Each milliliter of this solution was assumed to be the caloric equivalent of 0.2 gm. of sucrose in calculating the amount of sucrose required to keep the pair-fed controls in Group B isocaloric with the alcoholic animals in Group A.

Surviving animals numbered 1 to 25 were sacrificed by decapitation at the end of 7 days, the remainder at the end of 14 days. Both kidneys were removed, blotted dry, and weighed. Subsequently one kidney was sectioned sagittally, the other transversely. These were fixed in 10 per cent formalin, imbedded in paraffin, sectioned at 5 μ and stained with hematoxylin and eosin and a modification of the Masson trichrome stain. The livers were similarly weighed and analyzed for fat, as described in the preceding paper (1). A portion of the right lobe was saved for histological sectioning and staining.

The extent and severity of the renal cortical necrosis and medullary tubular changes were graded mild, moderate, or severe. With few exceptions the lesions in the two kidneys did not differ significantly. Tubular epithelial changes were not considered significant unless frank necrosis was present. The necrotic changes, which were best demonstrated in Masson-stained sections, were graded as mild if only a few of the proximal tubules were involved, moderate

[1] The authors are indebted to Dr. W. D. Salmon, Alabama Polytechnic Institute, Auburn, for carrying out this analysis.

if less than half the tubules were involved, and severe if more than half the tubules were involved. An attempt was also made to distinguish between the stages of active necrosis and healing. Many sections, of course, showed both. However, a lesion was considered to be in the healing stage when many of the necrotic epithelial cells had been removed, when the fibroblastic reaction and cellular exudation were at their height, when necrotic areas showed evidence of calcification, and when active mitotic activity could be demonstrated in the tubular epithelium. During the stage of active necrosis most of the moderate and severe lesions showed hemorrhage into the cortex and subcapsular areas, and protein precipitates within dilated medullary tubules. However, as healing progressed these vanished.

Results.—As is evident from Fig. 1, the incidence and severity of renal necrosis at the end of the first week were significantly greater in the alcohol-fed animals than in their pair-fed isocaloric controls. These differences were even more marked at the end of the 2nd week, by which time the alcoholic rats also had significantly heavier kidneys and a higher mortality rate. Since renal cortical necrosis and an increase in kidney weight are well recognized signs of choline deficiency in the weanling rat (3–5), these results may be interpreted with reasonable confidence as evidence that alcohol induced a state of relative choline deficiency, particularly since it was found in Experiment 2 that this effect could be prevented by supplementing the diet with choline. The fact that a few renal lesions were also demonstrated in both pair-fed control groups (B and C) indicates that the choline content of the basal diet was insufficient to fully protect the kidneys, so that the addition of alcohol merely magnified a partial deficiency state. That this effect was not due to an enhanced rate of growth or to a greater caloric intake is evident from a comparison of these measurements in Groups A and B (Table I). The possibility that the lower incidence of renal necrosis in the pair-fed isocaloric controls of Group B was related to a protective action of sucrose in the kidney rather than to abstention from alcohol can be excluded, since omitting the sucrose supplements from the diet in the pair-fed non-isocaloric controls of Group C did not increase the incidence or severity of kidney damage. Finally, it is highly improbable that the apparent increase in the incidence of renal necrosis following alcohol ingestion was an artefact due to the undetected resolution of lesions in the pair-fed controls of Groups B and C, since the relative proportions of healing and necrotic lesions in the three groups were very similar at the end of both the 1st and 2nd weeks (Fig. 2).

It will be noted that the *ad libitum* fed controls developed more severe signs of choline deficiency than either of the two pair-fed groups (Fig. 1). Since the former had a significantly higher caloric intake and gained more weight, at least by the end of the 2nd week (Table I), but did not differ from the latter in other respects, it is highly probable that their greater choline requirement was related to one or both of these factors. However, further studies are needed to establish this point, since the *ad libitum* fed controls also had a higher incidence of renal necrosis than either pair-fed group at the end of the

1st week (Fig. 1), at which time their caloric intake and weight gain were not significantly different (Table I).

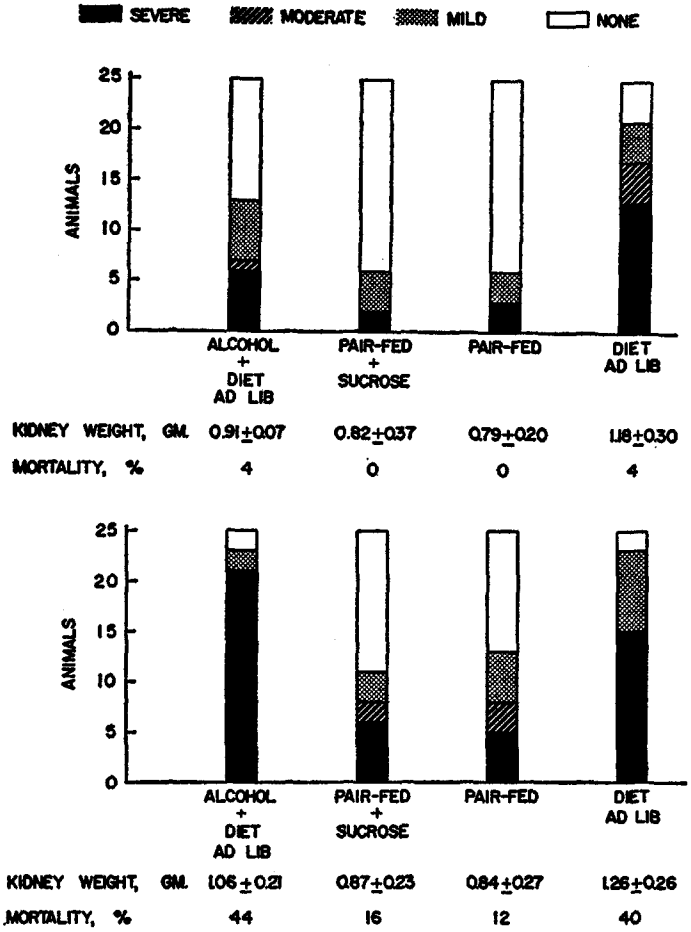

FIG. 1. Incidence and severity of renal necrosis at the end of 1 week (upper panel) and 2 weeks (lower panel). Statistically significant differences: Kidney weight—Group A (2 weeks) > B ($t = 3.0$, $p < 0.01$) and C ($t = 3.18$, $p < 0.01$); Group D (1 week) > A ($t = 3.56$, $p < 0.01$), B ($t = 3.70$, $p < 0.01$) and C ($t = 5.26$, $p < 0.01$); Group D (2 weeks) > A ($t = 2.94$, $p < 0.01$), B ($t = 5.43$, $p < 0.01$) and C ($t = 5.51$, $p < 0.01$); Mortality—Group A (2 weeks) > B ($\chi^2 = 4.66$, $p < 0.05$) and C ($\chi^2 = 6.36$, $p < 0.02$); Group D (2 weeks) > C ($\chi^2 = 5.08$, $p < 0.05$).

The fact that the alcoholic animals did not show more severe signs of choline deficiency than the *ad libitum* fed controls (Fig. 1) raises the question of whether the apparent increase in the incidence of renal necrosis in the alcohol-fed animals as compared to their pair-fed isocaloric controls, was not a statistical artefact related to an inadequate number of observations. However, this

is unlikely since the difference between these two groups has been confirmed in several other experiments involving larger numbers of animals (Table II). It is highly probable, therefore, that the induced state of choline deficiency was related to the ingestion of alcohol in this instance and to some other factor, possibly the caloric intake or rate of growth, in the case of the *ad libitum* fed controls.

TABLE I

Summary of Weight Gain, Daily Intake of Food, Alcohol, and Calories, and Hepatic Lipids

Experimental group*	Weight		Dietary intake	Alcohol intake	Caloric intake				Hepatic lipids
	Initial	Gain			Diet	Alcohol	Sucrose	Total	
	gm.	*gm.*	*gm.*	*ml.*‡					*gm. per cent*§
1 week									
A Basal diet and alcohol *ad libitum*	46 ± 3	17 ± 5	6.2 ± 1.1	6.9 ± 0.6	26 ± 7	5 ± 1	—	31 ± 5	19.3 ± 7.1
B Pair-fed and isocaloric (sucrose instead of alcohol)	46 ± 4	20 ± 3	6.2 ± 1.1	—	26 ± 7	—	5 ± 1	31 ± 5	20.6 ± 5.0
C Pair-fed	43 ± 5	19 ± 3	6.2 ± 1.1	—	26 ± 7	—	—	26 ± 7	17.1 ± 5.0
D Basal diet *ad libitum*	47 ± 3	20 ± 4	7.6 ± 1.4	—	32 ± 6	—	—	32 ± 6	17.6 ± 5.0
2 weeks									
A Basal diet and alcohol *ad libitum*	45 ± 2	26 ± 10	5.2 ± 1.3	6.7 ± 0.9	22 ± 7	5 ± 1	—	27 ± 6	17.9 ± 7.5
B Pair-fed and isocaloric (sucrose instead of alcohol)	46 ± 6	29 ± 5	5.2 ± 1.4	—	22 ± 6	—	5 ± 1	27 ± 7	19.9 ± 7.2
C Pair-fed	43 ± 6	25 ± 3	5.2 ± 1.3	—	22 ± 6	—	—	22 ± 6	10.4 ± 5.1
D Basal diet *ad libitum*	47 ± 5	36 ± 12	8.1 ± 2.3	—	34 ± 10	—	—	34 ± 10	16.4 ± 7.3

Statistically significant differences: Weight gain in 2 weeks D > A ($t = 2.33$, $p < 0.05$), D > B ($t = 2.10$, $p < 0.05$), D > C ($t = 5.78$, $p < 0.01$); daily total caloric intake (1 week) A > C ($t = 2.39$, $p < 0.05$), B > C ($t = 2.39$, $p < 0.05$), D > C ($t = 3.16$, $p < 0.01$); daily total caloric intake (2 weeks) D > A ($t = 2.20$, $p < 0.05$), D > B ($t = 2.38$, $p < 0.05$), D > C ($t = 4.3$, $p < 0.01$), A > C ($t = 2.50$, $p < 0.02$), B > C ($t = 2.65$, $p < 0.02$); hepatic lipids (2 weeks) A > C ($t = 2.20$, $p < 0.05$), B > C ($t = 3.02$, $p < 0.01$), D > C ($t = 1.82$, $p < 0.10$, > 0.05).

* 25 animals per group.

‡ 15 per cent ethyl alcohol.

§ On basis of wet weight.

When the results of the hepatic lipid analyses are considered (Table I), it is evident that the alcoholic group did not differ significantly from its pair-fed isocaloric or *ad libitum* fed controls. However, the fat content of the liver in the unsupplemented pair-fed controls of Group C was significantly lower than in any of the other groups at the end of 2 weeks, suggesting that when the caloric intake was lowered sufficiently the choline requirement for lipotropic activity in the liver was reduced. The data presented provide no satisfactory explanation for the fact that the relative choline deficiency induced by alcohol was not reflected in an increase in hepatic lipids. However, there is evidence to show that more choline is required to meet the demands for lipotropic activity in the liver than for protection against renal necrosis (3 *a*), so that if

the degree of fatty infiltration encountered in these experiments represented a maximal response, the effects of small changes in available choline may have been obscured.

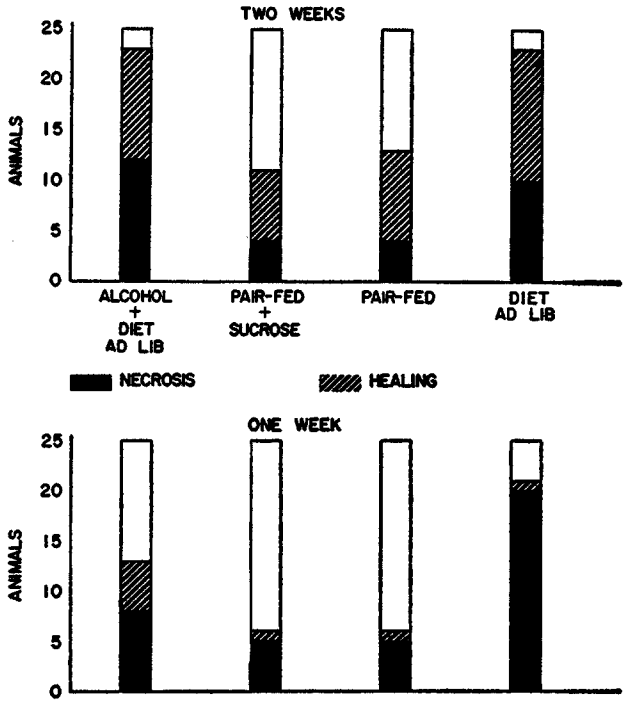

FIG. 2. Relative incidence of healing and necrotic lesions in the kidney

Experiment 2

In the following experiment an attempt was made to quantitate the increase in the choline requirement following alcohol ingestion.

Method.—One hundred and eighty Sprague-Dawley weanling rats were divided into 12 groups of 15 each, as follows:—

	Choline supplement			
	0.00 per cent	0.08 per cent	0.12 per cent	0.20 per cent
Basal diet and alcohol *ad libitum*	I A	II A	III A	IV A
Pair-fed isocaloric controls (sucrose *in lieu* of alcohol)	I B	II B	III B	IV B
Basal diet *ad libitum*	I C	II C	III C	IV C

The feeding and analytical technics and the basal diet were the same as in Experiment 1, except that choline supplements were incorporated in the diet as indicated above. All surviving animals were sacrificed at the end of 14 days.

Results.—The alcohol-fed animals maintained on the *unsupplemented* basal diet exhibited a higher incidence of renal necrosis (Fig. 3) and greater kidney weight (Fig. 4) than their pair-fed isocaloric controls, thus confirming the observations in Experiment 1 which led to the conclusion that alcohol increased the choline requirement but did not do so by augmenting the caloric intake. *Ad libitum* feeding of the same diet also produced more severe signs of choline

TABLE II

Mortality Rate and Incidence of Renal Necrosis in Rats Fed Alcohol and the Basal Diet ad Libitum for 14 Days Compared with That of Pair-Fed Isocaloric Controls

Experiment	Experimental group	No. of animals	Renal necrosis		Mortality	
			No.	Per cent	No.	Per cent
1	Alcohol and diet *ad libitum*	25	23	92	11	44
	Pair-fed isocaloric controls	25	11	44	4	16
2	Alcohol and diet *ad libitum*	15	13	87	5	33
	Pair-fed isocaloric controls	15	5	33	1	7
3	Alcohol and diet *ad libitum*	25	20	80	1	4
	Pair-fed isocaloric controls	25	8	32	3	12
4*	Alcohol and diet *ad libitum*	25	22	88	9	36
	Pair-fed isocaloric controls	25	9	36	1	4
5‡	Alcohol and diet *ad libitum*	25	19	76	5	20
	Pair-fed isocaloric controls	25	7	28	0	0
Totals....	Alcohol and diet *ad libitum*.....	115	97▲	84	31■	27
	Pair-fed isocaloric controls.....	115	40▲	35	9■	8

* Basal diet contained 10 μg. of vitamin B12 per 100 gm.

‡ Both groups of animals received an intramuscular supplement of vitamin B12 daily equal to that eaten by the animals in Experiment 4.

▲ Difference statistically significant: $\chi^2 = 58.8$, $p < 0.01$.

■ Difference statistically significant: $\chi^2 = 14.6$, $p < 0.01$.

deficiency than occurred in the pair-fed isocaloric controls, but in this instance the difference between the two groups appeared, as previously, to be related to the significantly higher food intake and greater gain in weight that occurred in the *ad libitum* fed animals (Table III).

As is evident from Figs. 3 and 4, the addition of as little as 0.08 per cent choline to the basal diet afforded complete protection against kidney damage, not only in both control groups but also in the animals receiving alcohol. This confirms the tentative conclusion reached in the previous experiment that the basal diet was suboptimal in choline content and that alcohol thus increased

the incidence of renal necrosis by magnifying a preexistent minor choline deficiency. From the mean food intake of the pair-fed isocaloric controls (Table III) it may be estimated that their daily choline intake was equivalent to

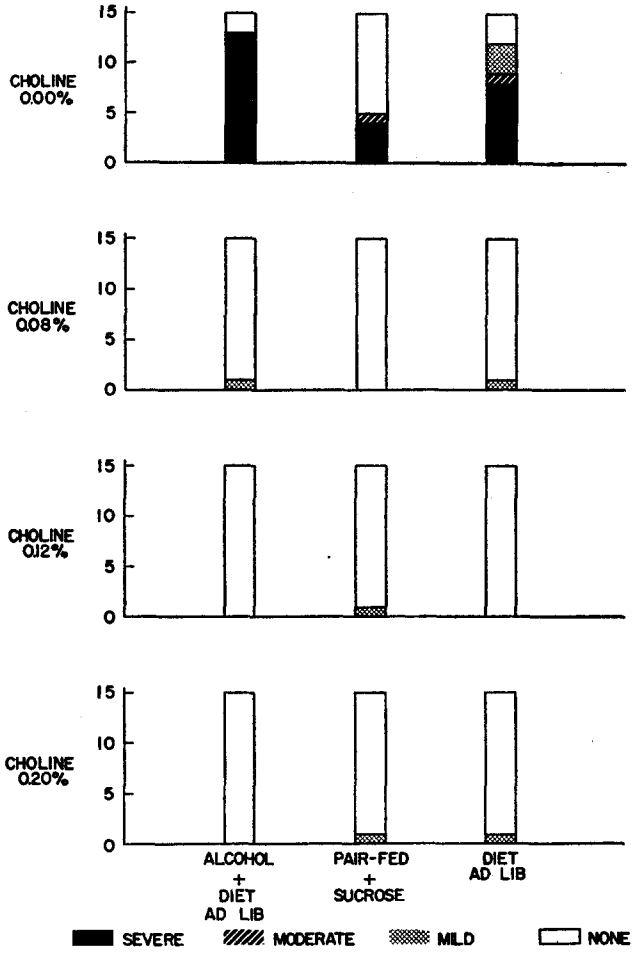

FIG. 3. Effect of choline supplements on the incidence and severity of renal necrosis at the end of 14 days.

4.0 ± 1.2 mg. on the basal diet, and 10.6 ± 2.4 mg. on the diet supplemented with 0.08 per cent choline. It is reasonable to assume, therefore, that the amount of choline they required to completely protect against renal necrosis, and the extent to which alcohol was capable of increasing this requirement, fell between these two values.

The results of the hepatic lipid analyses were similar to those obtained in

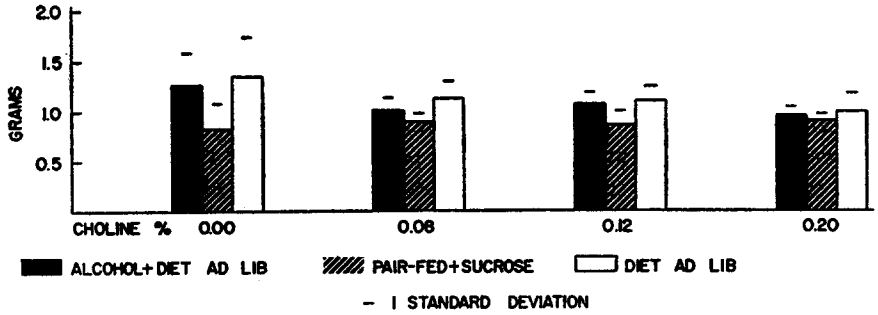

FIG. 4. Effect of choline supplements on kidney weight at the end of 14 days. Kidney weight in alcoholic animals on the *unsupplemented* diet significantly greater than in pair-fed isocaloric controls ($t = 4.21$, $p < 0.01$). Also, kidney weight in *ad libitum* fed animals on the *unsupplemented* diet significantly greater than in pair-fed isocaloric controls ($t = 4.45$, $p < 0.01$).

TABLE III

Summary of Weight Gain and Daily Intake of Diet, Alcohol, and Calories

Experimental group*	Weight		Dietary intake	Alcohol intake	Caloric intake			
	Initial	Gain			Diet	Alcohol	Sucrose	Total
	gm.	*gm.*	*gm.*	*ml.*‡				
Choline supplement—0.00 per cent								
I A Diet and alcohol *ad libitum*	61 ± 6	19 ± 6	5.0 ± 1.6	7.2 ± 2.0	21 ± 7	6 ± 2	—	27 ± 8
I B Pair-fed isocaloric controls	54 ± 5	17 ± 5	4.5 ± 1.4	—	19 ± 6	—	5 ± 1	24 ± 7
I C Basal diet *ad libitum*	61 ± 8	26 ± 12	7.5 ± 1.6	—	32 ± 7	—	—	32 ± 7
Choline supplement—0.08 per cent								
II A Diet and alcohol *ad libitum*	60 ± 6	38 ± 14	6.6 ± 1.4	9.8 ± 1.1	28 ± 6	8 ± 1	—	36 ± 6
II B Pair-fed isocaloric controls	54 ± 7	38 ± 5	6.3 ± 1.4	—	27 ± 6	—	8 ± 1	34 ± 6
II C Basal diet *ad libitum*	61 ± 7	56 ± 12	9.3 ± 2.0	—	39 ± 8	—	—	39 ± 8
Choline supplement—0.12 per cent								
III A Diet and alcohol *ad libitum*	59 ± 5	40 ± 11	6.6 ± 1.4	10.4 ± 1.3	28 ± 6	8 ± 1	—	36 ± 7
III B Pair-fed isocaloric controls	53 ± 4	32 ± 16	6.1 ± 1.3	—	26 ± 6	—	8 ± 1	34 ± 6
III C Basal diet *ad libitum*	64 ± 7	52 ± 18	9.1 ± 1.9	—	39 ± 8	—	—	39 ± 8
Choline supplement—0.20 per cent								
IV A Diet and alcohol *ad libitum*	58 ± 5	37 ± 8	6.2 ± 1.3	9.9 ± 1.0	26 ± 5	8 ± 1	—	34 ± 6
IV B Pair-fed isocaloric controls	59 ± 7	35 ± 6	6.1 ± 1.3	—	26 ± 6	—	8 ± 1	34 ± 6
IV C Basal diet *ad libitum*	52 ± 7	45 ± 23	8.1 ± 2.3	—	34 ± 10	—	—	34 ± 10

Statistically significant differences: Weight gain: I C > I B ($t = 2.50$, $p < 0.02$), II C > II A ($t = 3.68$, $p < 0.01$), II C > II B ($t = 5.0$, $p < 0.01$), III C > III A ($t = 2.08$, $p = 0.05$), III C > III B ($t = 2.73$, $p < 0.02$); Caloric intake: I C > I B ($t = 2.47$, $p < 0.05$).

* 15 animals per group.

‡ 15 per cent ethyl alcohol.

± values = standard deviation of the mean.

the previous experiment in that no significant differences were observed between the three groups at any given level of choline intake (Fig. 5). However, as might be expected, the highest values occurred in the groups maintained on the unsupplemented basal diet, while successively lower values were found as

the size of the choline supplement was increased. Judging from the absence of renal lesions, the smallest supplement used (0.08 per cent) was sufficient to overcome the deficiency of choline in the basal diet, both in the absence and presence of alcohol, so that no differences in hepatic lipids were to be expected between the alcoholic animals and their pair-fed isocaloric controls. However, if a sufficiently small supplement had been used to prevent renal necrosis in the pair-fed animals but not in the alcoholic group, it might have been possible to demonstrate the effect of alcohol on the choline requirement by a rise in the hepatic lipids of the latter group. Obviously further studies are needed to establish this point.

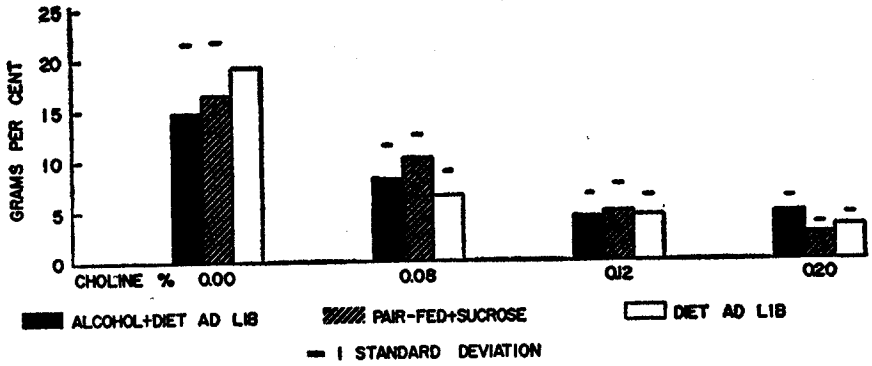

FIG. 5. Effect of choline supplements on hepatic lipids at the end of 14 days. Differences between groups at each level of choline not statistically significant.

DISCUSSION

The suggestion first made by Best and his associates (8), that alcohol increases the choline requirement of the rat and, thus, induces a deficiency state when the diet is marginal in lipotropic activity, is amply confirmed by the results of the present experiment and those reported in the preceding paper (1). They are not consistent, however, with the hypothesis put forward by these investigators that the alcohol effect is the consequence of adding calories to the diet, a theory based on the assumption that alcohol and sucrose supplements affect the liver in an identical manner since they both produce fatty infiltration. The data presented indicate that, although an increase in the caloric intake does magnify the signs of choline deficiency when the diet is relatively low in lipotropic activity, the deficiency state induced by alcohol ingestion is independent of the number of calories consumed.

Further studies are needed to elucidate the mechanisms underlying these effects. Certainly it is not clear whether it is the increased metabolic activity or the enhanced rate of growth which is responsible for the increased choline requirement when calories are added to the diet. Of particular interest is the

fact that the choline requirement appears to increase out of proportion to the number of additional calories consumed. In Experiment 1, for example, increasing the caloric intake without changing the choline:calorie ratio of the diet induced a deficiency state, as evidenced by the higher incidence of renal necrosis in the *ad libitum* fed animals than in the pair-fed unsupplemented controls (Fig. 1).

Little progress has been made in clarifying the mode of action of alcohol, but further application of the described technic for assaying changes in the choline requirement in the weanling rat should make more rapid strides possible. Suggestive evidence has been presented to show that alcohol does not interfere with the digestion, absorption or utilization of protein, choline, or methionine (1). However, it should be pointed out that the size of the dietary supplements used in these experiments was far in excess of the estimated increase in the choline requirement induced by alcohol ingestion, so that if alcohol partially blocked the absorption or utilization of these materials the effect may have been obscured. Since alcohol increases the choline requirement only to the limited extent demonstrated in these experiments, the additional demand for lipotropic substances is effectively met by relatively small supplements to the diet. No doubt this accounts for the long delay in recognizing the alcohol effect before Best and his associates (8) shrewdly employed a diet of marginal lipotropic activity to demonstrate it.

Even if it can be shown by the use of appropriately small lipotropic supplements that alcohol does not interfere with the absorption or utilization of lipotropic substances, a number of other possible mechanisms remain to be explored. These have been discussed at some length in another paper (9), and are now under investigation. Unfortunately up to the present our efforts to elucidate the mechanism underlying the alcohol effect on the choline requirement have not met with success.

The long debated question of whether alcohol is toxic for the liver has become a problem in semantics, now that it is recognized that toxic agents may exert their injurious effects on the liver by creating a relative deficiency of essential chemical compounds often derived from the diet (10). In that sense these agents may be said to induce a relative nutritional deficiency, especially since their effects, in some instances, may be abolished by appropriate dietary measures. It is clear from the evidence presented in this report and by Best *et al.* (8) that alcohol also induces a specific type of nutritional deficiency, so that in this respect its action resembles that of certain toxic agents.

There is little doubt that chronic choline deficiency in animals leads to a type of hepatic injury which closely resembles Laennec's cirrhosis in man (11, 12). While direct experimental evidence is still lacking, clinical observations in malnourished individuals and chronic alcoholics with Laennec's cirrhosis strongly suggest that the lesion in man is also, at least in part, related to a

deficiency of lipotropic substances. In the case of chronic alcoholism the deficiency is generally attributed to the inadequate dietary intake which often accompanies heavy drinking. Although the results of the present investigation provide confirmatory experimental evidence that alcohol ingestion leads to a reduction in food consumption, and, hence, to a decrease in available lipotropic substances, they also indicate that alcohol may compound this deficiency by increasing the choline requirement. Unfortunately, the elaborate pair-feeding technic required to demonstrate the latter effect in animals makes it difficult, if not impossible, to establish its importance in man. However, the following clinical observations suggest that the specific effect of alcohol on the choline requirement is probably of significance in the pathogenesis of Laennec's cirrhosis: (*a*) the withdrawal of alcohol and the maintenance of a suboptimal dietary intake often leads to improvement in the clinical, functional, and histological status of the liver in patients with Laennec's cirrhosis (13), (*b*) the incidence of Laennec's cirrhosis is higher in chronic alcoholics than in equally poorly nourished individuals with other diseases, and (*c*) Laennec's cirrhosis is occasionally seen in heavy drinkers whose dietary intake has been reasonably well maintained.

It is interesting to note, as pointed out in the preceding paper (1), that alcohol ingestion in rats leads to a reduction in food consumption in excess of the number of alcohol calories consumed, so that the total caloric intake falls below that of *ad libitum* fed animals. The reason for this response is unknown, but it is unlikely that it is due to anorexia related to any type of chronic gastritis, since it can be demonstrated as soon as alcohol feeding is begun. The problem is even more complex in man, since food consumption is reduced not only as a physiological consequence of ingesting alcohol calories, but also because of gastrointestinal disturbances, apathy, and economic problems stemming from excessive drinking.

Although the emphasis in this discussion has been on the importance of nutritional factors in the relationship between chronic alcoholism and Laennec's cirrhosis, it is not our intention to imply that other factors have necessarily been excluded. Indeed, there is a need for a careful investigation of the possible effects of alcohol on the regenerative capacity of the liver, and on its susceptibility to injury during infection.

SUMMARY

The effect of alcohol on the choline requirement was assayed in weanling rats maintained on a basal diet of relatively low lipotropic activity containing the equivalent of 0.089 per cent choline. Alcohol was administered as a 15 per cent solution *in lieu* of drinking water. The incidence of renal cortical necrosis, the increase in kidney weight, and the mortality rate at the end of 14 days served as indices of choline deficiency. Under these conditions alcohol-fed

animals developed more severe signs of choline deficiency than either pair-fed controls or pair-fed *isocaloric* controls receiving a sucrose supplement instead of alcohol. The addition of as little as 0.08 per cent of choline to the basal diet abolished these differences. It was concluded that (*a*) alcohol increases the choline requirement, and may, thus, induce a state of relative deficiency when the diet is marginal in lipotropic activity, and (*b*) this effect is independent of the caloric intake. The possible significance of these observations in relation to chronic alcoholism in the pathogenesis of Laennec's cirrhosis has been discussed.

BIBLIOGRAPHY

1. Klatskin, G., Krehl, W. A. and Conn, H. O., *J. Exp. Med.*, 1954, 605.
2. Barrett, H. M., Best, C. H., MacLean, D. L. and Ridout, J. H., *J. Physiol.*, 1939, **97**, 103.
3. Griffith, W. H. and Wade, N. J., (*a*) *J. Biol. Chem.*, 1939, **131**, 567; (*b*) 1940, **132**, 627.
4. Christensen, K., *Arch. Path.*, 1942, **34**, 633.
5. Hartroft, W. S., *Brit. J. Exp. Path.*, 1948, **29**, 483.
6. Strength, D. R., Schaefer, E. A. and Salmon, W. D., *J. Nutrition*, 1951, **45**, 329.
7. Richter, C. P., *J. Exp. Zool.*, 1926, **44**, 397.
8. Best, C. H., Hartroft, W. S., Lucas, C. C. and Ridout, J. H., *Brit. Med. J.*, 1949, **2**, 1001.
9. Klatskin, G., *Yale J. Biol. and Med.*, 1953, **26**, 23.
10. Drill, V. A., *Pharmacol. Rev.*, 1952, **4**, 1.
11. Daft, F. S., Sebrell, W. H., and Lillie, R. D., *Proc. Soc. Exp. Biol. and Med.*, 1941, **48**, 228.
12. Blumberg, H., and McCollum, C. V., *Science*, 1941, **93**, 598.
13. Klatskin, G., and Yesner, R., *J. Clin. Inv.*, 1949, **28**, 723.