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THE INTERACTION BETWEEN DIETARY PROTEIN AND ALCOHOLISM:

I. Effects On Hepatic Lipids.

By

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INTRODUCTION

It has been established that protein malnutrition produces fatty liver both in human subjects and in experimental animals (Thomas, 1974). On the other hand, fatty liver has been considered one of the characteristic changes associated with human alcoholism despite adequate diet (Leiber et al., 1965). This change has also been reproduced in experimental animals.

Controversy continues, however, of whether this lesion is the result of a direct hepatotoxic effect of alcohol (Reubner et al., 1972) and (Nair et al., 1975); or that ethanol could alter nutrition by interfering with intestinal absorption of protein (Barboriak and Meade, 1969) and (Porta et al., 1970).

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In the present investigation, an attempt has been made to characterize the role of dietary protein and alcoholism as Co-factors in the development of fatty liver.

MATERIAL AND METHODS

Male Albino guinea pigs, of the strain bred and brought up in the serum and antigen Laboratory farms-Helwan, averaging 530 grams of body weight at the commencement of the experiment were used. They were divided into four groups according to the scheme in table (1).

Table (1)

Experimental design and group distribution

Group N°.	Experimental Design
Group I	High Protein diet & Alcohol administration
Control I	High Protein diet.
Group II	Low Protein diet & Alcohol administration
Control II	Low Protein diet.

The experimental diets, which were fed ad-libitum, consisted of:

Table (2)

Composition of diets

Ingredient	% Composition	
	High Protein diet	Low Protein diet
Casein	25%	5%
Corn Starch	62%	82%
Cotton Seed Oil	7%	7%
Mineral Mixture (Jones and Foster, 1942)	4%	4%
Vitamin Mixture (Morcos, 1967)	2%	2%

Alcohol was administred orally to groups I and II daily; for six days per week, at adosage level of C.6 ml./Kg./day.

Animals from each group were autopsied at the end of the second and fourth weeks. Fresh liver weights were determined. The total lipid content was estimated from 5 gram liver samples using a Soxhlet apparatus, following the method of Frazer (1949). The lipid was then dissolved in benzene and the free fatty acids (FFA) were estimated by the method of Varley, (1969).

For histological examination, liver samples were fixed in 10% formole-saline. Frozen sections were cut at 10 microns, and stained with Sudan IV for lipids.

RESULTS

The effect of different treatments on hepatic weights, total lipids, and free fatty acids is shown in table (3).

When liver weight data were expressed in terms of percentage per body weight, the diet showed no significant effect on liver weight. Alcohol administration reflected marked increase in liver weight. This was rather aggravated by the protein deficient diet.

Analysis of the livers for total lipids revealed marked elevation in both groups receiving ethanol. The low protein diet alone increased the hepatic lipids by only 2 : 3 fold; compared with that of the high protein diet.

The increase in free fatty acids in guinea pigs administering alcohol was marked at two weeks. A similar increase was brought up later at four weeks by protein deficiency. When ethanol was administered with low protein diet, FFA showed drastic rise, approximating three times of the normal.

Histological examination:

The livers of guinea pigs fed low protein diet revealed moderate to marked increase in intra-cellular lipids. (Figure 1) This was most marked in the central

Table (3)

Mean Values of liver weight, total lipids and free fatty acids:

Experimental design	Duration	Liver weight	Total lipid	Free fatty acids
	weeks	% per body wt.	% per fresh liver	mg./1 gm. Liver
High Protein diet + Alcohol (Group I)	2	3.47%	5.44%	31.4
	4	3.19%	7.74%	39.1
High Protein diet (Control I)	2	2.67%	1.97%	21.7
	4	2.27%	1.70%	21.5
Low Protein diet + Alcohol (Group II)	2	3.66%	9.07%	39.3
	4	3.80%	9.81%	52.0
Low Protein diet (Control II)	2	2.62%	3.70%	21.0
	4	3.16%	5.76%	34.8

portions of the liver lobules.

Alcohol adminstration when associated with a high protein diet developed severe fatty metamorphosis of periferal and mid lobular hepatocytes. About 40 to 50 per cent of the cells composing hepatic lobules were affected by small intracellular particles and large extracellular fatty cysts (Figure 2).

When alcoholism was super-imposed on low protein diet, the livers from animals of this group demonstrated extensive fatty metamorphosis. Large extracellular lipid particles occupied 75 to 80 per cent of the hepatocytes throughout the liver lobules (Figure 3).

DISCUSSION

The present investigation indicated that ethanol adminstration developed acute fatty liver without a quantitativly important malabsorption of fat, and despite adequate diet. Inhibition of intestinal transport of nutrients by ethanol (Leiber et al. 1969) did not seem to increase losses of fat from the intestine during the development of alcohol - induced fatty liver.

On the basis of the amount and distributional pattern of hepatic lipids in protein deficiency, it is suggested that the lesion is in its early stage. Severe fatty metamorphosis similar to that produced by alcohol adminstration seem to require prolonged periods of protein depletion (Kosterlitz, 1947).

In comparing the dietary effect with ethanol adminstration, it was evident that 80% decrease in protein intake doubled hepatic lipid, whereas a moderate dose of ethanol increased it 2 : 3 fold in a similar duration. Consequently, it is proposed that hepatic response to ethanol is ~~immediate~~ and severe. In connection with this finding, several investigators have reported that a single dose of ethanol can produce fatty liver in experimental animals, (Di Luzio et al., 1958), Strubelt, 1972) and (Nair et al., 1973). This has either been attributed to an enhanced mobilisation of FFA from the adipose tissues through an adrenal hypophyseal pathway (Haling et al., 1960). Other studies have emphasized the direct hepatotoxic effect of alcohol leading to hepatic steatosis (Klatskin, 1961). The possibility that alcohol may induce a relative lipotropic

deficiency is suggested by Huebner et al., (1972). If this is so, then it might account for the present finding that hepatic steatosis in alcoholism was accentuated by additional protein deficiency.

ABSTRACT

Albino guinea pigs were given ethanol orally, and hepatic lipid response was determined biochemically and histochemically in the presence of high or low protein diets.

The investigation indicated the following:

1. Analysis of the livers for total lipids and free fatty acids revealed marked elevation in both groups receiving ethanol. The low protein diet alone reflected moderate increase.

2. Histochemical study of rat demonstrated generalized fatty metamorphosis of extracellular lipid when ethanol was administred with alow protein diet. With a high protein diet, periferal and midlobular fatty infiltration developed, while the low protein diet reflected a moderate increase in intracellular lipids in a similar duration.

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Figure (1)

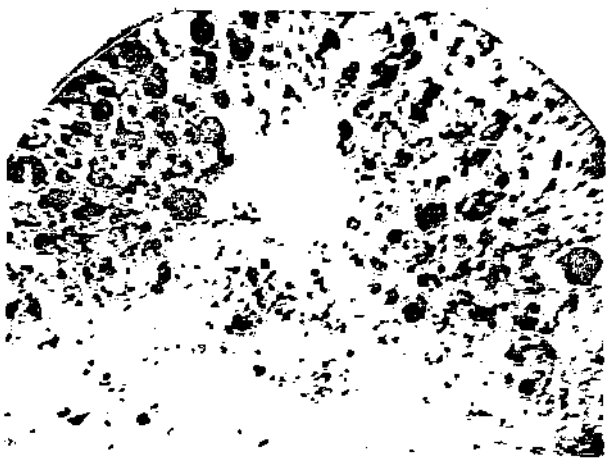
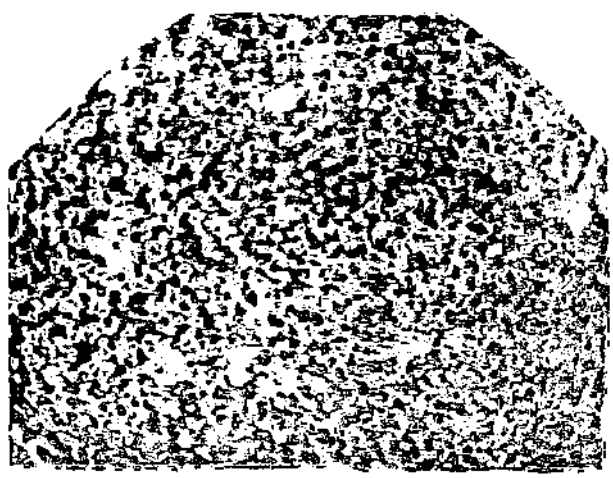


Figure (2)

Figure (3)



Captions of Figures

(Frozen Sections, Sudan IV, X 125)

Figure (1): 4 weeks on alow protein diet. Intracellular lipid particles.

Figure (2): 4 weeks of alcohol adminstration and a high protein diet. Large Lipid particles in the mid-lobular hepatocytes.

Figure (3): 4 weeks of alcohol adminstration and alow protein diet. Generalized fatty metamophosis.