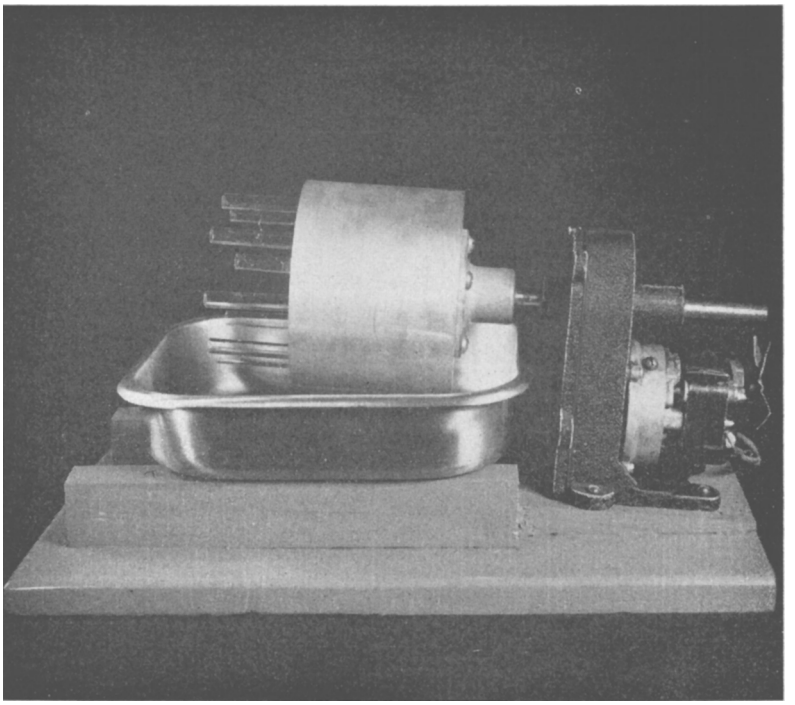

FIG. 2.
A machine for shell-freezing biological materials in small glass ampoules.

16099

## Production of Fatty Infiltration of Liver in Rats by Alcohol in Spite of Adequate Diet.

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The part played by alcohol in liver cell injury and cirrhosis has not been conclusively established. Prior to the recognition of the influence of dietary factors in liver injury, many observations were made in an attempt to ascertain the solution to this question. Moon[1] summarized and critically evaluated these reports, concluding that there was no definite evidence that alcohol itself produced liver cell injury. At the time of Moon's review, 1934, the dietary factor was not given any consideration, and its significance was not apparently recognized. Lamson and Wing[2] showed that the combination of alcohol with carbon tetrachloride increased the severity and incidence of liver cell injury and cirrhosis. This observation was evidently acceptable to both sides of the question as a compromise and seemed to constitute the basis for prevailing opinion on the subject until recent years. Following the elucidation of the dietary factor in the problem of liver cell injury,[3,4,5]

[1] Moon, V. H., *Arch. Path.*, 1934, **18**, 381.

[2] Lamson, P. D., and Wing, R., *J. Pharm. and Exp. Therap.*, 1926, **29**, 191.

[3] Allen, F. N., Bowie, D. J., McLeod, J. J. R., and Robinson, W. L., *Brit. J. Exp. Path.*, 1924, **5**, 75.

[4] Best, C. H., and Huntsman, M. E., *J. Physiol.*, 1935, **83**, 255.

and the recognition of certain deficiency syndromes in the chronic alcoholic patient,[6,7] there has been a progressive tendency to attribute all of the organic changes in alcoholism to the supposed universal dietary deficiency.[8]

The observations of Connor,[9] however, are in opposition to this and should be noted. In rabbits, he was able to produce fatty infiltration, liver cell atrophy, necrosis of liver cells, and fibrosis in variable degree by daily administration of alcohol, even though a diet with high casein content and an adequate amount of other known required factors were provided. The use of the rabbit, an animal in which perilobular fibrosis and parasitic disease of the liver are common findings, the fact that the animals did not take an adequate amount of the diet, and the lack of control animals, make it difficult to assess fully the findings in Connor's experiment.

The problem has therefore been undertaken in rats. Adult female rats of the Sprague-Dawly strain were used. Four series were employed:

Group I—Low casein diet,* and alcohol,
Group II—High casein diet, and alcohol,
Group III—Low casein diet, no alcohol, and
Group IV—High casein diet, no alcohol.

The administration of alcohol caused a reduction of food intake. The amount of food administered was therefore placed under control by allowing Groups I and II to eat *ad libitum,* and the amount taken was determined by weighing the food containers. The amount of food given to Group III and IV was the same as that eaten by the corresponding group taking alcohol, and on the preceding day. Alcohol was administered daily by stomach tube, the amount of 1.5 cc of 25% alcohol per 100 g, and 10% alcohol was given as the only drinking water. Approximately 0.6 cc of absolute alcohol per 100 g of body weight was taken daily by these animals. The regime was continued for about 2 months, and at the end of this time the animals were autopsied and histological study of the livers and other organs made.

*Results.* The rats receiving alcohol became obviously intoxicated and not infrequently were comatose for short periods following the administration of alcohol. These animals ate poorly. The groups receiving no alcohol continued to exhibit good appetite throughout, although the animals receiving low casein diet later became slightly less inclined to eat their food.

The weight declined in all groups. Fig. 1 and 2 show the decrease for each animal.

Fatty livers were found in every animal of Groups I, II and III. No fatty change was found in Group IV. The gross and histological findings were characteristic of this degenerative lesion. The liver was increased moderately in weight and was somewhat yellowish and friable. Histological study of hematoxylin-eosin and Sudan stained sections revealed fatty deposition in the liver cells and was moderate or marked, predominantly the latter. No evidence of cirrhosis was found.

*Discussion.* In these experiments, the effect of emaciation from quantitative nutritional lack has been eliminated by providing the same amount of food to the corresponding control and alcohol groups. The weight curves further establish this point, since the group receiving alcohol and adequate diet, showed slightly less weight decline than those receiving no alcohol and quantitatively adequate diet. Thus any liver cell injury might well

5 Du Vigneaud, V., *Biol. Symposia,* 1941, **5**, 234.

6 Alexander, Leo, *Am. J. Path.,* 1940, **16**, 61.

7 Goodheart, R., and Jolliffe, N., *Am. Heart J.,* 1938, **15**, 569.

8 Wright, A. W., *Arch. Path.,* 1941, **32**, 670.

9 Connor, C. L., *Arch. Path.,* 1940, **30**, 165.

* The composition of the diets[10] employed was:

| | Low casein | High casein |
|---|---|---|
| Lard | 40% | 40% |
| Agar | 2 | 2 |
| Starch | 48 | 28 |
| Salt mixture | 48 | 28 |
| Osborne, Mendel | 5 | 5 |
| Casein | 5 | 25 |

Each animal was given daily one drop of Vi Penta furnished through the courtesy of Hoffmann-LaRoche), each 0.6 cc of which contains 5000 USP units of vitamin A, 1 mg of thiamine HCl, 1 mg of riboflavine, 50 mg of ascorbic acid, 1000 USP units of activated ergosterol, and 2 mg of niacinamide.

10 Tidwell, H. C., and Treadwell, C. R., *J. Biol. Chem.,* 1946, **162**, 155.

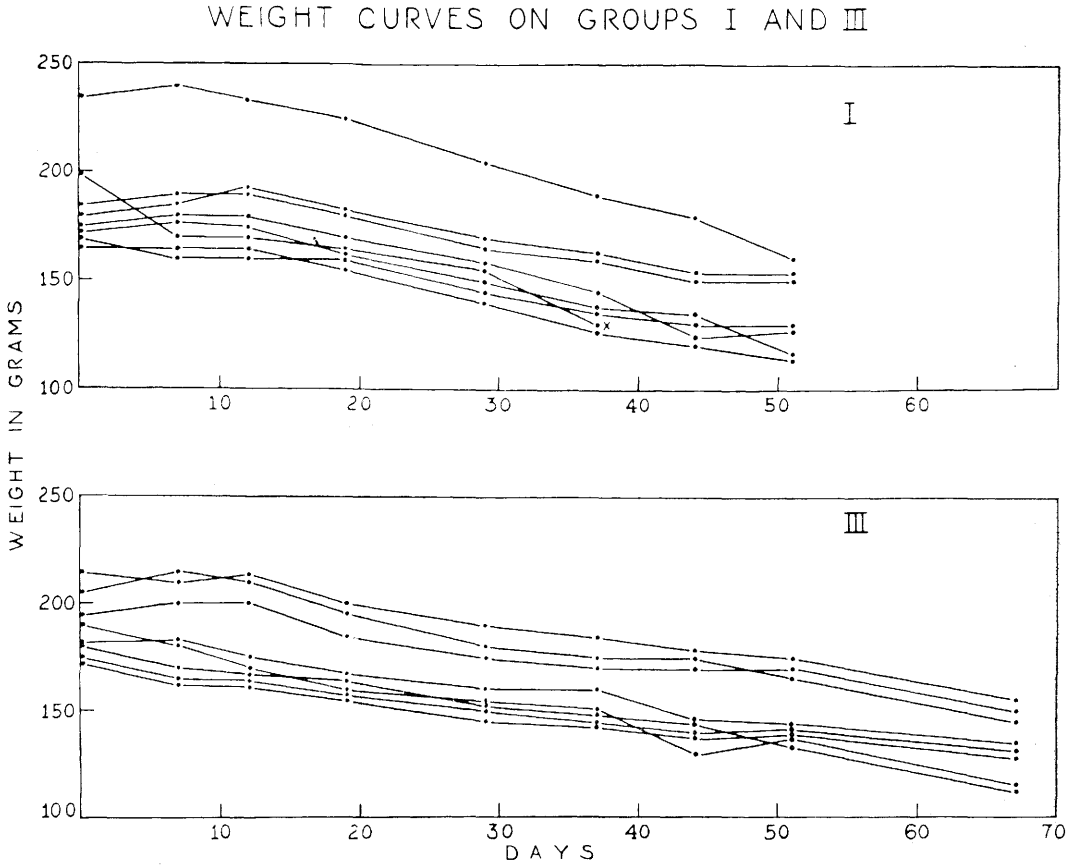


FIG. 1.

be attributed to the only apparent variable, namely the presence or absence of alcohol. The fact that in Group II, receiving high casein diet and alcohol, fatty infiltration of the liver occurred in all animals, would seem therefore to indicate that alcohol itself does produce liver cell injury, and in this instance, fatty infiltration. There was no apparent difference in the incidence or severity of fatty degeneration nor is there any histological difference in the character of the lesion in the animals receiving alcohol and high casein diet, as compared to those receiving low casein diet with or without alcohol. The duration of the period of observation was too short to determine the occurrence of cirrhosis in these animals. It remains to be shown whether the liver cell injury produced by alcohol is due to a direct toxic action, to the effect of the degradation products of alcohol, or whether alcohol interferes with the chemical processes normally called into play in the metabolism of fat.

*Summary.* Rats were given alcohol daily and either a high or low casein diet Control series received similar diets and no alcohol. The amount of food intake in control groups was regulated according to the amount taken by the rats receiving alcohol. The effect of total food intake in those receiving alcohol as compared with those not receiving alcohol was further observed by weight curves. Fatty infiltration of the liver occurred in every animal receiving alcohol and low casein diet, alcohol and high casein diet, and in all those receiving low casein diet and no alcohol. None of the animals receiving the same volume of the high casein diet and no alcohol had fatty infiltration of the liver. It is therefore concluded that alcohol exerts an effect

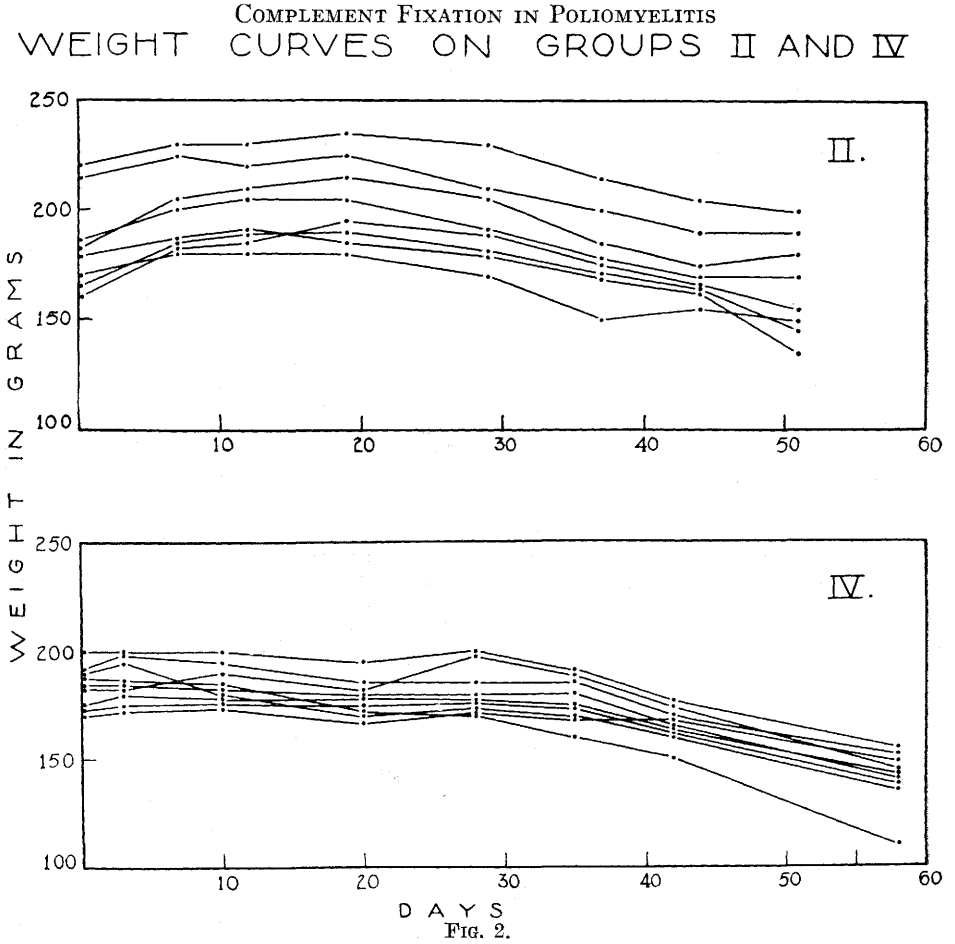


FIG. 2.

which permits accumulation of fat within the liver cells, and that this effect operates separately from that of extrinsic deficiency of lipotropic factors.

## 16100

## Complement-Fixation in Experimental and Human Poliomyelitis.*

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Although many attempts have been made to find a simple immunological means of studying poliomyelitis, only the neutralization reaction has yielded consistent and reliable results. Possible reasons for the lack of success of precipitin and complement-fixation tests have been discussed by several authors.[1] The possibilities include (a) low reactivity or avidity of the neutralizing antibodies, (b) small size of the virus particle, and (c) insufficient concentration of virus in the antigen

* Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

1 Schultz, E. W., Gebhardt, L. P., and Bullock, L. T., *J. Immunol.*, 1931, **21**, 171; Raffel S., and Schultz, E. W., *J. Immunol.*, 1940, **39**, 256.