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The Influence of Diet, Environment and other Factors on Experimental Liver Necrosis in the Rat

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This paper deals with factors affecting the sensitivity of rats to the development of acute dietary liver necrosis. The sensitivity is influenced by the way the diet is fed (to appetite or restricted) as well as by the chemical composition of the diet, the environment, the age at weaning and other factors.

Chemical composition of the diet

Weichselbaum (1935) was the first to report acute damage ('haemorrhages') in the liver as a result of feeding a low-protein diet to rats. The diet was similar to that described by Sherman & Merrill (1925) and contained dried whole-milk powder 16·6, corn (maize) starch 80·6 and salt mixture 2·8%. Four drops of cod-liver oil and 75–150 mg Marmite were given to each rat daily. Sherman & Merrill did not report liver lesions or deaths.

During the course of the next few years several independent reports from America, Britain and Germany described acute liver necrosis in the rat as the result of feeding poor diets. By 1949 it was generally believed that deficiency both of sulphur-containing amino-acids and of vitamin E was necessary to produce the lesions. The former deficiency was usually achieved by feeding rats on diets low in casein (5–10%) (Schwarz, 1944; Himsworth & Glynn, 1944–5; György & Goldblatt, 1949; Hove, Copeland & Salmon, 1949) or on diets in which the sole source of protein was yeast (Hock & Fink, 1943; Himsworth & Glynn, 1944–5; Schwarz, 1948; Abell & Beveridge, 1949). However, György & Goldblatt (1939, 1949) found that liver necrosis either did not appear or was reduced in incidence when a yeast diet was fed, or when yeast was added to their casein diet. It has now been suggested that these discrepancies were due to differences in the yeast used (György, Rose, Tomarelli & Goldblatt, 1950; György & Goldblatt, 1951; Schwarz, 1951, 1952). That caseins also differ in their capacity to induce liver necrosis has been reported by Schwarz (1944), Hove, *et al.* (1949), and Naftalin (1954*b*); this topic is discussed later in this paper.

György & Goldblatt (1949) suggested that liver necrosis could be produced

with greater certainty if unsaturated fat were added to the deficient diet; Abell & Beveridge (1949, 1951) showed that rancid fat and cod-liver oil increased the 'necrogenic' quality of the diet. (For further details see Dam, 1953.)

Environment and manner of feeding

From a survey of the work up to 1949 it appeared to me that two other variables were worthy of further study, namely the way the diet was fed and the environment in which the experiments were made. Male albino rats about 38 days old were studied in a range of environmental temperatures. They were either allowed to eat to appetite or were restricted in their food intake to a calculated amount of that eaten by a group of their litter-mates. The same semi-synthetic diets without added vitamin E were fed to every rat (Naftalin, 1951, 1952, 1954*a*). The results showed that the optimum conditions to produce necrosis were an environmental temperature of 70–80°F in which the rats ate to appetite. Whether a rat lived or died of liver necrosis or died of inanition depended not on the amount of food eaten *per se* but on the degree of food restriction in relation to the environmental temperature. Thus at environmental temperatures of about 60°F and about 90°F with food restricted to 80% of that eaten by the group fed to appetite, the rats lived to the end of the experimental period (70 days). In an environment of 70–80°F there was no difference in incidence of liver necrosis between those rats fed to appetite and those restricted to 80%; nearly all the rats died of liver necrosis. At 70–80°F even a high degree of food restriction—60% of the amount eaten by the group fed to appetite in the same environment—did not prevent the development of liver necrosis in every rat in the 'restricted' group. One-quarter of these 'restricted' rats died from liver necrosis: the rest survived. The same degree of food restriction (60%) at about 60°F or about 90°F resulted in deaths without liver necrosis. At about 40°F or below a smaller degree of food restriction (70–80%) resulted in deaths without liver necrosis.

It should be noted that these results were obtained with young albino male rats weaned on their 24th day of age, and managed in the manner previously described (Naftalin, 1951, 1952). Although the trends are clear, the exact degree of food restriction to prevent necrosis at any given environmental temperature may be different with other methods of management. No observations have been made of meteorological variables other than dry-bulb air temperatures.

In warm environments of above 70°F, rats fed to appetite were inactive and sluggish. They tended to lie in a corner with snouts down and backs humped. By contrast rats fed to appetite in the cold (below about 45°F) or rats in any environment, whose food intake was so restricted as to prevent necrosis, were active and continually jumping about. Their patterns of behaviour suggested that metabolism in the various groups was different (Naftalin, 1951) and the metabolic aspect of the problem requires further study. These experiments and other evidence (György & Goldblatt, 1949; McLean & Beveridge, 1952; Gillman, Gilbert, Gillman & Spence, 1952) seriously question the view that acute liver necrosis may be regarded simply as combined deficiencies of cystine (or methionine) and vitamin E.

Factors other than experimental diet and environment

One of the earlier difficulties in investigating the lesion was inability to produce it at will. Even if a 'necrogenic' diet is fed to rats in the optimum environment for the production of the disease, the results may be variable. The following factors modify either the incidence of liver necrosis or the time taken for necrosis to develop after the 'necrogenic' diet is fed.

Sex. Weichselbaum (1935) and Abell & Beveridge (1951) observed that young male rats are more sensitive than young females to the development of dietary liver necrosis, but Gillman *et al.* (1952), by using a very 'necrogenic' diet of food yeast and cooked potato starch, were unable to demonstrate a sex difference in sensitivity.

Age or initial weight. Young rats of about 35–55 g are more sensitive than older rats of 120 g or more. Rats 21 days of age given the experimental diet develop the lesion in a shorter time than those given the diet when 40 days old or more (Wahi, 1949; Lindan & Himsworth, 1950; Abell & Beveridge, 1951; Goettsch, 1951; Gillman *et al.* 1952).

Pre-experimental diet. The supplementation of the pre-experimental diet with vitamin E or with cystine increases the time subsequently taken to produce the disease or decreases the incidence of the disease (Himsworth & Lindan 1949; Lindan & Himsworth, 1950; Goettsch, 1951).

Mother's diet. The young of mothers fed on a diet low in vitamin E are more sensitive to the development of liver necrosis than those of mothers whose diet contain an adequate amount of vitamin E (Goettsch, 1948, 1951; Lindan & Himsworth, 1950).

Weaning age. In seeking to enhance sensitivity and also to provide a means of comparing sensitive groups of rats with groups less sensitive to the development of dietary liver necrosis, the effect of weaning litter-mates at different ages was investigated (Naftalin, 1954*c*). Groups of rats weaned early, on the 17th day, developed liver necrosis in a shorter time than groups of their litter-mates weaned on the 25th day. The differences between rats weaned early and late may possibly be accentuated if large litters are used. The procedure I now adopt to obtain groups for comparison is to allow the mother to suckle eight rats till the 17th day of lactation, then to remove six leaving two sucklings till the 25th day. To equalize the groups for experimentation, only two of the rats weaned early are kept.

Fig. 1*a* shows the result of an experiment to test the effect of age at weaning; the differences between rats weaned early and late are marked. Similar results have been obtained in nine out of the ten experiments done to date. Fig. 1*b* shows the result obtained in the one experiment of the series when even the rats weaned early did not exhibit a high degree of sensitivity. In the experiments illustrated the same batches of ingredients, except for the sucrose which was from two different samples, were used in the semi-synthetic diets. The rats were the inbred hooded Lister strain of the Rowett Institute stock. The mother's diet was Rowett Institute stock cubes together with cow's milk till the 17th day of lactation.

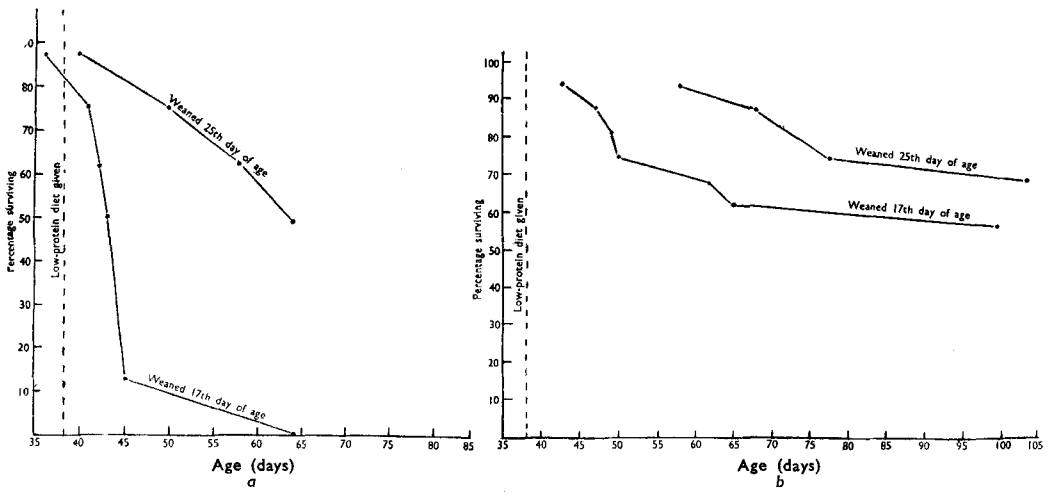

Fig. 1. Relation of age at weaning to development of dietary liver necrosis in rats. (*a*) Eight rats per group. Mortality: early weaning 100%, late weaning 50%. (*b*) Sixteen rats per group. Mortality: early weaning 44%, late weaning 25%.

In both experiments the rats were fed from the 17th to the 37th day of age on a diet containing 16% Glaxo casein 'C' (unextracted); from the 38th day the casein was reduced to 8%. The same batches of all ingredients were used in both experiments with the exception of the sucrose. The mothers received the Rowett Institute stock cubes and cow's milk till the 17th day of lactation.

All deaths were due to acute liver necrosis. The survivors were killed when 110 days old.

Variations in the mothers' diet may explain the differences between the results of these two experiments, but I suggest that all the factors upon which sensitivity to the development of dietary liver necrosis depend are not yet known and controllable. These results also illustrate the inadvisability of comparing the effects of different treatments in separate experiments. Urgent problems are to define accurately wherein lie the differences between rats sensitive and those slightly less sensitive, and the mechanisms governing these differences.

Pregnancy. Studies of acute dietary liver necrosis have usually been made on young rats or young adult rats. However, Goettsch (1949) noted that pregnant rats fed on diets low in vitamin E and in sulphur-containing amino-acids may suffer liver necrosis. Lindan (1951) studied the effects of rearing female rats on diets low in vitamin E. When mated and fed on a 'necrogenic' yeast diet those rats that became pregnant developed acute liver necrosis towards the end of pregnancy. The rats that did not become pregnant though similarly reared, mated and fed on the 'necrogenic' diet did not develop liver necrosis.

Genetic variation. Little work has been done on this aspect of the problem but there is the possibility that strain differences exist; the differences observed between litters of the same strain may possibly be due to a genetic factor.

Nature of caseins and yeasts used in the diet

Schwarz (1944, 1952) and Hove *et al.* (1949) reported that crude casein contained a factor that prevented the development of liver necrosis in rats

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fed on low-protein, vitamin E-deficient diets. Schwarz (1951) has shown that his factor, which he has named 'factor 3' because he calls vitamin E and cystine 'factors 1 and 2', is present in some American-grown yeasts, e.g. cultured primary dried yeast and debittered dried brewer's yeast (Anheuser-Busch Inc., St. Louis), but not in American-grown torula yeast (Lake State Yeast Corporation). This finding of Schwarz possibly explains the difference between the results of the early work of György & Goldblatt (1939, 1949) and that of Himsworth & Glynn (1944–5), and of Hock & Fink (1943) in that there may have been differences in the content of 'factor 3'. The 'necrogenicity' of a yeast is not related to the lowness of its content of cystine (György *et al.* 1950; Lindan & Work, 1951*a*,*b*; Gillman *et al.* 1952).

In the earlier part of my work the 'vitamin-free' casein of Glaxo Laboratories Ltd was used. When this casein was fed as 8% of a suitable diet a high incidence of liver necrosis resulted in rats housed at 70–80°F and fed to appetite. Under the same conditions when a batch of the casein of 'low-vitamin' content of Genatosan Ltd was used liver necrosis did not result. It was then found that Glaxo casein 'C' (unextracted)—a crude casein—was 'necrogenic'. On inquiry from the manufacturers it was found that Glaxo caseins are mainly of New Zealand origin and Genatosan caseins are made mainly from British milk.

Samples of crude caseins were therefore obtained from New Zealand and caseins extracted with alcohol and ether were made from dried skim milk from New Zealand, England or Northern Ireland. These preparations have been tested using litter-mate rats weaned on their 17th day of age (Naftalin, to be published). Caseins of New Zealand origin whether crude or alcohol-ether extracted were 'necrogenic'. Caseins prepared from English or Irish milks were associated with a low incidence of liver necrosis; this result was obtained even when rats known to be highly sensitive to the development of liver necrosis were used.

The reason for the difference between these caseins may possibly lie in differences in content of the casein 'co-factor' of Hove *et al.* (1949), or of 'factor 3' of Schwarz (1951, 1952), but until these factors are isolated and the amounts estimated one must bear in mind the possibility of another explanation.

SUMMARY

The composition of diets capable of producing liver necrosis is described. The most commonly used 'necrogenic' diets are those with 5–10% of casein or 10–18% yeast as the source of protein, devoid of vitamin E and containing rancid or unsaturated fat. The nature of the casein or the yeast influences the 'necrogenicity' of the diet.

Whether or not dietary liver necrosis results when rats are fed on a 'necrogenic' diet depends on the interaction between the environment and the way the diet is fed (to appetite or not). Other factors such as age, sex, age at weaning, the pre-experimental treatment, and pregnancy, affect sensitivity to the development of acute liver necrosis.

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Storage of Vitamins in Liver

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I propose to describe as storage that amount of vitamin laid down in excess of the actual physiological level needed for normal functioning of the organ. When using this criterion it can be seen that the vitamins fall roughly into three groups. (1) Those that are used by the tissues to be built into coenzyme systems and are precursors of prosthetic groups of enzymes. I shall call them prosthetins. Their level in the liver does not appear to exceed a certain saturation point, which most likely is the saturation of apoenzymes by their respective prosthetic groupings.