A REVIEW OF THE DIETETIC FACTORS IN LIVER DISEASE

BY

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I.—DIETARY CIRRHOSIS AND NECROSIS OF THE LIVER*

Two distinct disorders of the liver have been produced in animals by defective diets. The first is a fatty infiltration of the liver which proceeds slowly to cirrhosis of the portal type. The second is acute necrosis, which, if not fatal, is followed by post-necrotic scarring and nodular hyperplasia. The two lesions may sometimes commingle, and not all workers would agree that they can be sharply differentiated.

Fatty Infiltration and Cirrhosis of the Liver

Soon after insulin was discovered it was observed that pancreatectomized dogs kept alive by diet and insulin tended to develop severe fatty infiltration and eventual cirrhosis of the liver, which could be prevented by feeding them with pancreas. Experiments showed that lecithin also was effective, and likewise choline, which is one of the components of lecithin. The effect of pancreas is probably not entirely explained by lecithin, for pancreas contains another substance called lipocaic, which has an action in discharging fat from the liver. Fatty infiltration and cirrhosis can also be produced in intact animals by giving a high-fat diet, and subsequent work has for convenience been done mostly on small animals such as rats and mice. The process is a slow one, but after an interval of several months a progressive fibrosis of the portal type develops, presumably because the excess of fat interferes with the nutrition of the liver cells. It can be checked at any time by lecithin or choline, which bring the fat content of the liver back to normal. The maintenance dose of choline is probably of the order of 1 mg. per 100 g. body weight of rat per day, though it is customary to give ten or twenty times this amount, and high dosage is necessary for curative purposes.

Later it was found that the addition of adequate amounts of casein to the diet prevented fatty infiltration, and it was a natural step from this to discover that fatty infiltration could be produced by reduction of the protein, even in the absence of a high fat intake. Whereas casein prevents fatty infiltration, gelatin is relatively ineffective, and the action of casein appears to be explained by one of its component amino-acids, methionine. Choline and methionine have in common the possession of methyl (CH_3) groups, which are fairly loosely attached—in the one case to nitrogen, in the other to sulphur atoms. These labile methyl groups are very important in the economy of the body. When there is a deficiency of labile methyl groups the body is unable to manufacture lecithin, which is required for the transport of fat, and the fat therefore accumulates in the liver and eventually damages it. Cystine, which resembles methionine in containing sulphur but contains no methyl groups, has

no curative action in fatty infiltration, though it is effective against acute necrosis. Substances which encourage the normal transfer of fat from the liver are called lipotropic (McHenry and Patterson, 1944), and the series I have mentioned is the most important:

Pancreas → lecithin → choline → labile methyl group
Casein → methionine →

Fatty infiltration of the liver in the pancreatectomized animal is probably due to the absence of an enzyme, perhaps identical with lipocaic, which liberates methionine from combination and makes it available as a lipotropic factor (Chaikoff, Entenman, and Montgomery, 1945).

Acute Necrosis of the Liver

When allowance was made for the lipotropic action which proteins share with choline owing to the possession of labile methyl groups, it was possible to show that protein deficiency may also act in a quite different and specific way and produce acute necrosis of the liver (Himsworth and Glynn, 1944a). In contrast to the gradual development of fatty engorgement and cirrhosis of the liver over a period of months, acute necrosis comes on suddenly in the midst of apparent health after an interval of several weeks on the defective diet. The time of onset and the severity of the attack depend mainly on the degree of deficiency of the diet. Different proteins vary in their ability to prevent necrosis; whereas casein is effective in small amounts, yeast protein is ineffective in large amounts. The main difference between the two proteins is that yeast is poor in amino-acids containing sulphur, and in fact the protective value of different strains of yeast has been shown to vary in proportion to their nitrogen-sulphur ratios.

The two thio-amino-acids are methionine and cystine. By feeding rats on synthetic diets in which the nitrogen was supplied in the form of pure amino-acids it has been shown that necrosis will not develop if an adequate amount of cystine is supplied (Glynn, Himsworth, and Neuberger, 1945). Cystine can be replaced by methionine, which is converted into cystine in the body. These results suggest that the essential factor in preventing dietary necrosis is the sulphhydryl (SH) group, just as the methyl (CH_3) group is the essential factor in preventing dietary cirrhosis. The broken link in the metabolic chain in sulphhydryl deficiency is presumably cystine, whereas the broken link in methyl deficiency is phospholipin. Methionine, which contains both methyl and sulphhydryl groups, is a key substance in the metabolism of the liver, and it is one of the indispensable amino-acids. There is evidence that an excess of cystine may be harmful. Glynn and his co-workers obtained protection with 30 mg. per rat per day, whereas Earle and Victor (1942) found that 300 mg. per rat per day produced lesions which were apparently of the necrotic type. This

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last figure would correspond with a human dose of 180 g., so that ill-effects need not be feared in therapeutic experiments in man.

It should be noted that yeast does not actively favour necrosis: it is merely poorly protective. Yeast is a good source of choline, and it possibly contains other unknown lipotropic principles (György and Goldblatt, 1939; Rich and Hamilton, 1940). Yeast may therefore be expected to be a useful remedy in most forms of fatty infiltration of the liver due to dietary imbalance. Some of the B vitamins, particularly biotin, favour the deposition of fat in the rat's liver; this type of fatty liver is resistant to choline but responds to lipocaic or inositol. In fact, the relation of the B vitamins to cirrhosis and necrosis of the liver has not yet been completely elucidated. Growing rats and mice have a high rate of metabolism, which affects their vitamin B requirements, and in larger animals the action of the B vitamins on the liver may be different (Fouts, 1943). On the present evidence it seems a justifiable conclusion that no ill effects on the liver are likely to accrue from massive dosage with yeast or other sources of the vitamin B complex in hepatic disease in man.

Diet and Resistance to Hepatitis

In view of the gross damage to the liver which may result from dietary defect it is not surprising that lesser deficiencies may impair the resistance of the liver to injury by poisons. It has been known for many years that animals which have been starved or been fed on an excessive proportion of fat develop fatty infiltration of the liver, and with this goes a diminished resistance to a number of hepatic poisons. It has also long been known that a damaged liver contains little glycogen, and that animals fed on high-carbohydrate diets are in general less susceptible to drugs which produce accumulation of fat in the liver than are those given high-fat diets (Rosenfeld, 1903). Not only does a high carbohydrate intake give some protection against poisoning by chloroform and phosphorus but it is also of value in the prevention of hepatic damage following experimental ligation of the common bile-duct, operation of Eck's fistula, partial hepatectomy, and experimental mushroom poisoning (Soskin and Hyman, 1939).

For carbohydrate to be effective it is probably necessary that glycogen should be deposited in the liver, that fat should be displaced, and that the ingested carbohydrate should spare the body stores of protein. It is an unfortunate error to think that the mechanical displacement of fat by glycogen is the whole story. The truth is rather that carbohydrate is the fuel for the hepatic cell in its work of fat transport, protein synthesis, and detoxication. The great value of carbohydrate may well be that it spares protein. Carbohydrate is not entirely comparable to the petrol in the internal combustion engine, as it may be used to neutralize poisons by forming conjugates with them, but its action is mainly as a source of energy which is quickly consumed. Carbohydrates cannot protect the liver unless the requirements of protein metabolism and total calories are satisfied. If these precautions are not observed an unbalanced carbohydrate regimen may actually favour the development of necrosis (Craven, 1931).

Attention was first concentrated on the part played by protein in maintaining the resistance of the liver when Davis and Whipple (1919) demonstrated that not only carbohydrate but also protein would protect the starved dog against chloroform poisoning. Much of the subsequent work of Whipple and his group has been done with dogs depleted of protein by special diets and sometimes by withdrawal of plasma as well. They have shown that injury to the liver from chloroform increases as protein stores decrease, and that a protein meal before anaesthesia is

beneficial (Miller and Whipple, 1940). They demonstrated that the sulphur-containing amino-acids, methionine and cystine, are the specific protective factors and that methionine is more effective than cystine. Finally Brunswick and his co-workers (1945) showed that equal protection was given by the non-nitrogenous compound thioglycollate, and the only common factor was the simple sulphhydryl (SH) group. While chloroform damages the liver of the normal dog, with the organic arsenicals the dog must first be depleted of protein, or the animal will die from the other toxic effects of the arsenic before the liver is attacked. Here again the protective action of protein has been demonstrated and traced to the methionine level.

There are fewer experiments on the resistance of animals with choline deficiency, presumably because it has so long been known that the fatty liver is vulnerable and also because fatty infiltration may only slowly disappear under specific treatment. Best and his co-workers (Best *et al.*, 1935; Barrett, 1939) have shown that when rats are maintained on a diet low in lipotropic factors and then poisoned with phosphorus or carbon tetrachloride the addition of choline to the diet has no effect on the degree of fatty infiltration or damage to the liver cells, but it speeds up the rate of disappearance of fat from the liver in the recovery phase.

This brings us to the time factor in protection experiments, which is of special importance when we come to apply the results to man, for in man we can rarely take action till exposure has occurred. Methionine will give complete protection against chloroform if administered shortly before anaesthesia. It will give partial protection three to four hours after anaesthesia, but no protection more than four hours after anaesthesia (Miller and Whipple, 1942). With mapharside protection has been obtained by the oral administration of methionine 20 to 24 hours before the injection of the arsenical, but only partial protection with intravenous injection just prior to mapharside injection. The latter failure may well be due to too small a dose—1 g. per dog, instead of the 3 g. used in the other experiments (Goodell *et al.*, 1944). Most of the work so far recorded has dealt with animals on defective diets. A variety of food factors, including methionine, glucose, para-amino-benzoic acid (Sandground and Hamilton, 1943), liver extract, and sodium xanthine (Barrett *et al.*, 1938), will enhance the normal resistance against certain poisons, but there is no evidence that choline can act in this way.

Dietary Treatment of Experimental Hepatitis

The lipotropic action of protein and the antinecrotic action of the protein sulphur lead us to expect that protein would be of great value in the treatment of hepatic injury, and at first sight it is puzzling to find that apparently it is not. Treatment is a different thing from prophylaxis. Much of the experimental assessment of therapy has been carried out on animals poisoned with carbon tetrachloride. Some of the factors affecting recovery from carbon tetrachloride poisoning have been carefully studied by Lamson, Minot, and Robbins (1928). They found that dogs treated with carbon tetrachloride more commonly died from acute necrosis when they were on a lean-meat diet than on a mixed diet containing bone-meal. The addition of calcium to the preceding diet of lean meat reduced the mortality to normal. Acute necrosis after carbon tetrachloride in man is more common in children and in the malnourished, and they suspect that this is due to calcium deficiency. Lamson (1930) recalled that Whipple had found that a high-carbohydrate diet protects dogs against poisoning by chloroform, and he pointed out that such a diet usually takes the form of bread-and-milk, which is high in calcium and low in meat.

The high-calcium diet does not diminish the liver damage: it merely diminishes the symptoms. These symptoms are attributed to a high blood level of guanidine, which leads to severe hypoglycaemia and peripheral circulatory failure. Calcium salts neutralize the guanidine, and glucose repairs the hypoglycaemia. On this argument death in hepatitis may be due to incomplete metabolites or products of autolysis, and if these can be neutralized spontaneous recovery may occur. Lamson (1930) further showed that the feeding of meat to susceptible animals causes a tremendous increase in blood guanidine, with a fall in blood sugar, tetany, convulsions, gastro-intestinal haemorrhage, and death. Guanidine has always been something of a will-o'-the-wisp in metabolic diseases, and this line of research has seemingly been held up by the necessity for studying its pharmacology. Certainly Bollmann and Mann (1936) found no excess of guanidine in the experiments described in the next paragraph. The relief of the symptoms of acute necrosis of the liver by calcium recalls recent work on adenosine triphosphate, which is believed to be an important factor in shock from tissue damage. Adenosine triphosphate is potentiated by magnesium, and magnesium and calcium are physiological antagonists (Green and Stoner, 1944).

Bollmann and Mann (1936) have experimented with diets in a variety of hepatic injuries in dogs. After performance of Eck's fistula a condition known as meat intoxication is liable to develop, though the name perhaps exaggerates the extent to which it is due to meat. After complete obstruction of the bile-duct a diet rich in carbohydrate becomes increasingly necessary for the maintenance of the animal. After two or three months a diet exclusively of meat will prove fatal within a week, but if the diet is high in carbohydrate life may be prolonged for at least a year. The animals are extraordinarily prone to develop ascites after feeding on meat. The active substance which induces ascites is probably not protein, because milk protein does not favour ascites, whereas meat extract produces results which are even more striking than those of meat. Comparable results were obtained with 16 dogs, each of which was given 10 ml. of carbon tetrachloride daily by mouth. The four dogs that were given a high-fat diet all died within three weeks. Of the four dogs that were given an entirely meat diet, two died within three months, and the other two had ascites. The remaining eight dogs, which were given mixed diets, were all alive at the end of three months, though the four which had received their protein in the form of meat were not in such good shape as those which had received milk.

Bollmann (1943) has more recently determined the time of survival of rats which were exposed to carbon tetrachloride vapour for 30 minutes three times a week. On the ordinary mixed diet the rats lived from 36 to 42 days. In a second group of experiments half of this diet was replaced with isocaloric values of cracker-meal and sucrose, meat, or lard. Expressing the normal survival time as 100, the investigators obtained the following figures for survival: carbohydrate, 146; protein, 100; fat, 91. In another series three-fourths of the mixed diet was replaced with isocaloric values of the above foodstuffs, and the protective values were now: carbohydrate, 205; protein, 104; fat, 59.

Dietary Cirrhosis of the Liver in Man

A low intake of protein with insufficient supply of the vitamin B complex, including choline, is a prominent feature of the diet of persons addicted to alcohol. In the earlier stages of alcoholism fatty infiltration of the liver is said to occur, and there is a growing tendency to affiliate alcoholic cirrhosis with the cirrhosis produced in animals by lack of lipotropic factors. But alcohol is not the only

cause of cirrhosis in man, nor is it the commonest. All writers on tropical pathology have laid stress on the frequency of liver cirrhosis with ascites among native races, where the influence of alcohol can be discounted (Manson-Bahr, 1945).

References to cirrhosis in India, Ceylon, and China are given in Ratnoff and Patek's (1942) review. A striking feature of tropical cirrhosis is the high incidence in children and the relative frequency of recovery, even when the disease has reached the ascitic stage. The hepatic lesions have generally been attributed to the interplay of malnutrition and infection, and no clear line has been drawn between portal cirrhosis and postnecrotic fibrosis. In Jamaica, where infantile cirrhosis of the liver is common, it has been noted particularly in the children of mothers who, generally being unmarried, have to support themselves and their babies by working in the urban areas or on plantations or elsewhere. Breast-feeding, therefore, is scanty, and the infant's diet consists mostly of carbohydrate—e.g., arrowroot, cereal gruel, or even sugar and water. There is a suggestion that the disease is less frequent in fishing villages, where protein intakes may be higher (McFarlane and Branday, 1945; Platt, 1946).

Throughout Africa there occurs a condition known as infantile pellagra, or "kwashiorkor." It is found in infants, and to a less extent in adults, who are fed on bulky vegetarian low-protein diets, and it is characterized clinically by oedema, pellagrous skin lesions, steatorrhoea, and macrocytic anaemia (Trowell and Muwazi, 1945). At necropsy or biopsy an extreme degree of fatty infiltration of the liver is found (Smith, 1943), and there is no doubt that this fatty liver is eventually followed by cirrhosis. Gillman (1944) noted that cirrhosis and cancer of the liver are especially prevalent among South African negroes, and he showed that cirrhosis and necrosis of the liver occurred in rats who were fed on the South African native diet. He concluded that recurrent attacks of malnutrition resulted in progressive hepatic damage. Subsequently the Gillmans (1945) showed by serial liver punctures of patients that the fatty infiltration responded slightly to liver extract and was completely cured by desiccated stomach in a dosage of 10 g. a day. It seems unlikely that the lipotropic action of desiccated stomach is entirely due to its choline content. With the technique of liver puncture and sternal puncture, and the introduction of pure factors such as choline, methionine, and folic acid, this whole field is now wide open to research, and we shall not have long to wait for the first harvest.

Dietary Protection Against Hepatic Disease in Man

It has been believed for many years, and probably with reason, that carbon tetrachloride and arsenicals are more likely to damage the liver when the patient is undernourished, but no precise data are available. Beattie (1943-4) has claimed that during antisyphilitic treatment with arsenical drugs the incidence of liver damage can be reduced significantly and the time of greatest liver damage can be postponed by the administration of casein digests, cystine, and methionine. Experiments of this kind are complicated by the virus of homologous serum jaundice and by the reduction of the antispasmodic activity of the arsphenamine by the sulphur compounds.

During the war the low incidence of infective hepatitis in coloured troops and native races was the subject of frequent comment, but less notice was taken of the high mortality they suffered. I have previously mentioned an outbreak in Belgian colonial troops, with a mortality of 4% (Witts, 1944). In 1943 a very high mortality was recorded in a small epidemic among some African labourers in Uganda who were in a poor state of

nutrition; of 14 cases admitted to hospital, all died.¹ Figures on the incidence and mortality of infective hepatitis in India Command during 1944 were collected by Lieut.-Col. Stuart McDonald.¹ These figures showed the usual difference between officers and other ranks. In addition they showed that the incidence of jaundice was five times as high in British troops as in Indian troops, but, on the other hand, the mortality was five times as great in the Indians as in the British. In the final issue British troops and Indian troops had an equal chance of dying of jaundice. There is some evidence that while malnutrition favours bacterial infection it antagonizes infection by viruses (Foster *et al.*, 1944). There was a good deal of anaemia and malnutrition among Indian troops.^{2,3} One cannot say whether the low incidence in Indians was due to nutritional factors or to previous exposure, but there is a strong suggestion that the relatively high death rate was due to malnutrition.

Discussion

A survey of the animal experiments leaves us in little doubt as to the importance of the lipotropic factors and the thio-amino-acids to the health of the liver. An excessive intake of fat will be harmful to the liver unless it is balanced by a corresponding increase in lipotropic factors, while a high-carbohydrate regimen helps by sparing the reserve stores of protein in the liver and providing easily available energy. Proteins supply the essential methyl and sulphydryl groups, but they may also contain harmful residues which are badly tolerated in acute and chronic liver disease. Milk proteins appear to be innocuous in this regard, whereas meat and meat extracts are harmful. Choline and lecithin are present in most animal and plant tissues, and the thio-amino-acids are particularly well represented in the proteins of milk.

The most important lipotropic factors are those which provide the labile methyl groups for the manufacture of phospholipins, without which fat transport comes to a standstill. Excess of fat in the liver leads to a gradual pressure atrophy of the cells and portal fibrosis, and it also accentuates the effect of the many toxic substances which are more soluble in fat than in water. Tropical cirrhosis and alcoholic cirrhosis of the liver in man appear to be due mainly to a deficient intake of lipotropic factors, but in both disorders a toxic factor is still suspected by many people.

The effects of a deficiency of protein sulphur are more subtle and profound. The sulphydryl groups are attached to the vital enzyme systems in the liver. Protein starvation depletes the liver of these sulphur-rich components, and if the process goes far enough the cell must die. Methionine and cystine are rapidly incorporated in the depleted liver to make good the deficiency. Chloroform and arsenicals disturb the enzyme systems, presumably by combining with or neutralizing the sulphydryl groups. The disturbance is reversible within the first three or four hours of administration of the poison, but after that methionine is no longer effective, and it is clear that viable liver cells are essential for the rapid uptake of methionine. Chloroform combines with the sulphydryl groups to form the non-toxic mercapturic acids. The action of arsenicals has been reviewed by Peters, Stocken, and Thompson (1945), who showed that they have a selective action upon the pyruvate enzyme system and that an essential sulphydryl component of the protein constituent of the system is attacked. As a result of their studies they introduced the dithiol compound

BAL, which prevents the toxic action of arsenic and some of the heavy metals if given before the administration of the poison and neutralizes it if the toxicity is not too long established.

Himsworth and Glynn (1944b) in their paper on toxipathic and trophopathic hepatitis have tried to integrate these data, and have suggested that in the final issue hepatic necrosis is always nutritional in origin, always due to a deficiency of essential factors. This deficiency may be primary, or it may be secondary to the increased metabolism, the swelling of the liver, and the disturbance of the circulation which result from infection or poisoning of the liver. The argument is an ingenious one, but it does not get us very far. The liver can be protected against poisons such as chloroform and arsenic by simple chemicals such as sodium thioglycollate and BAL, which act as rather specific antidotes, and it is unlikely that the results obtained with chemical poisons can be transferred without change to the action of biological toxins and viruses. In the latter conditions thio-amino-acids may act, if they act at all, by providing sulphydryl groups attached to the appropriate amino-acid residue for re-synthesis of essential sulphur proteins which have been inactivated, rather than by neutralizing the noxious agent.

[The second lecture, with a list of references, will appear in our next issue.]

OBSERVATIONS ON PLAGUE MENINGITIS

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Meningeal involvement in plague has occasionally been recorded, the earliest reports apparently being by the German Indian Plague Commission of 1897 and the Austrian Commission of the same year. These are cited by Meyer, Connor, Smyth, and Eddie (1937) in their review of most of the recorded cases of plague meningitis up to that year, while they discuss in detail their own case of "chronic relapsing latent meningeal plague" in California occurring in a patient whose illness began with an axillary bubo. Since then further cases have been reported, including one from Uganda by Burton and Hennessy (1940) of "subacute or chronic plague terminating in haematogenous meningeal infection," and four cases of plague meningitis complicating bubonic plague from South America by de Villafañe Lastra and Rodeiro (1942).

A particularly rare clinical form—primary plague meningitis—was reported from Dakar by Lafont, Lecomte, and Heckenroth (1915). Williams (1934) had a similar case in East Africa; and Lewillon, Devignat, and Schoetter (1940) reported still another from the Belgian Congo. These cases, like the one described in this paper (Case VIII), presented the common clinical features of meningitis with no bubo formation. In China Pollitzer (1946) has observed meningeal involvement in most recent outbreaks. In this connexion, therefore, it seems worth while to record eight cases of meningitis in a series of 203 cases of plague admitted to the General Hospital, Chuanchow, South China, during the years 1943–5.

Symptomatology

In all except one of our cases (No. VIII) meningeal involvement was a complication of pre-existing bubonic plague, symptoms appearing at the earliest on the ninth and at the latest on the seventeenth day of the disease. The clinical features closely resembled those of cerebrospinal fever or other acute meningitis. Headache, painful stiffness of the neck, and Kernig's sign appeared early and prominently. Special symptoms included convulsions (Cases III, VIII), cranial-nerve affections

¹ Unpublished reports to M.R.C. Jaundice Committee.

² Report on Investigations on Anaemia in the Indian Soldier from the Anaemia Investigation Team, G.H.Q., India, 1943–5; New Delhi, 1945.

³ Proceedings of the Central Command Conference on Anaemia, held at Agra on Nov. 20 and 21, 1944; Benares, 1945.