DIETETIC HEPATIC LESIONS AND PROTEIN DEFICIENCY*

M. V. RADHAKRISHNA RAO, N. C. DATTA AND L. S. KRISHNAN

(Department of Nutrition and Experimental Pathology, Haffkine Institute, Bombay)

AS a result of recent researches, a new orientation has been given to the etiology and treatment of liver diseases in general and 'cirrhosis of liver' in particular. Experimental evidence has accumulated to suggest that 'cirrhosis of liver' may be a deficiency disease. Malnutrition is widely prevalent in India and the incidence of 'cirrhosis of the liver' is fairly high in certain parts of the country. It was pointed out by the senior author (1936) that "dietetic deficiencies appear to be the important factors in the causation of the disease".

In view of its practical importance, a study of 'cirrhosis of the liver' both in its clinical and experimental aspects of the problem, was undertaken under the auspieces of the Indian Research Fund Association, in the Haffkine Institute, Bombay, since July 1944.

Experimental work was designed to study the effect of protein deficiency, with and without supplements of the vitamins of the B_2 complex on the histological and biochemical changes in the liver of rats, while the clinical investigation was mainly concerned with the evaluation of the effect of treatment of decompensated portal cirrhosis with high protein, high caloric diets with vitamin supplements.

1. Experimental Hepatic Cirrhosis

(a) *Effect of Vitamin B_2 Complex deficient diet with different levels of protein on the liver:* Casein was the main source of protein and the basal diets contained varying levels of the protein, namely 18, 10, 8 and 5 per cent. casein respectively. Animals in this group were deprived of all the vitamins of the B_2 complex. A total of 108 rats were used in this experiment in the different groups.

The growth and survival of the rats depended largely on the amount of casein in the diets. On the 5 per cent. casein diet, the animals did not survive for more than 2 to 3 months. In the other groups, the average life of the animals increased as the casein in the diet was raised.

The relationship between liver weight and body weight of the rats was found to vary according to the level of casein in the diets. In general, as the percentage of casein in the diet was decreased, the average liver weight per 100 g. body weight was increased.

The estimation of water, fat and protein content of the liver showed that this increase in the weight of the livers in the low protein groups was not due to accumulation of fat alone; for, there were significantly larger amounts of water and protein present as compared to normal liver.

(b) *Histological findings:* In animals receiving 18 to 10 per cent. casein diets respectively, the liver parenchyma showed moderate to severe fatty infiltration, particularly around the central veins. Traces of ceroid pigment was also visible in these areas. In the 8 per cent. and 5 per cent. groups, extensive fatty infiltration, more marked around the central veins was seen. An interesting feature in the 8 per cent. protein group was the appearance of replacement fibrosis, patchy in distribution around the central veins. In certain cases this was more marked and there was a tendency for the formation of pseudolobules. Animals receiving 5 per cent. protein diet did not show any marked replacement fibrosis. None of the animals in any group showed massive necrosis.

(c) *Control diets:* The composition of these diets was similar to the above and likewise the level of casein in diets varied from 5 to 18 per cent. respectively; the only difference being that each animal received in addition 0·5 g. of dried brewer's yeast per day as a source of the vitamins of B group.

In this group there was no significant difference in the amount of water, fat and protein content of the liver, except in the 5 per cent. group which showed slight increase in the water and fat content.

Histological findings

Animals receiving 18 and 10 per cent. casein diets with supplements of yeast showed normal structure of the hepatic parenchyma, while those receiving 8 and 5 per cent. casein showed slight fatty infiltration around the central veins. Fibrosis of

* A paper read before the Medical and Veterinary Section of the 36th Session of the Indian Science Congress, held at Allahabad in January 1949.

the liver was altogether absent in these groups of animals even after prolonged feeding of the diets.

2. Effect of restricted intake of protein [casein] in an otherwise adequate diet on the histological and biochemical changes in the liver of rat.

An interesting feature of the previous experiment was the absence of massive necrosis of the liver on protein-deficient diets. Himsworth and Glynn (1944) however, have claimed to have produced massive hepatic necrosis and its sequence in rats by limiting the intake of casein between 200-500 mg. per rat per day. They found that the amount of fat, carbohydrate, minerals or vitamins in the diet did not have any influence on the production of hepatic necrosis.

It was therefore thought worthwhile, to study the histological and biochemical changes in the liver of rats on restricting the daily casein intake at different specific levels in an otherwise adequate diet.

The average results are given below :—

Daily casein intake	Initial weight in g.	Final weight in g.	Calories in take	Liver wt. g./100 g. body wt.	Water g./100 g body wt.	Fat g./100 g. body wt.	Protein g./100 g. body wt.	Ash g./100 g. body wt.
(*a*) 1·2 g.	37·8	170·3	32·66	3·24	2·28	0·17	0·65	0·05
(*b*) 0·6 g.	37·5	149·5	28·6	2·87	2·04	0·17	0·55	0·04
(*c*) 0·4 g.	36·0	139·0	26·7	3·16	2·19	0·31	0·51	0·04
(*d*) 0·2 g.	38·8	89·0	19·3	4·16	2·81	0·48	0·65	0·05

The experiment was continued for more than 150 days. The growth of the animals paralleled the daily casein intake. The animals receiving a maximum of 1·2, 0·6 and 0·4 g. casein daily were quite healthy and normal and there were no signs of vitamin deficiency in any case, whereas those receiving a maximum of 0·2 g. casein showed considerable emaciation, loss of hair over the body and in general appeared weaker. There was no edema or ascites in any case.

From the results of the liver analysis given in the above table, it will be seen that the liver weight per 100 g. body weight shows a definite increase as the daily intake of casein was reduced. The amount of liver fat per 100 g. body weight also showed an appreciable increase as the intake of casein was progressively reduced.

The results of the total plasma protein concentration and the choline content of the liver of rats in the different groups are shown below :

Daily protein (casein) intake	1·2 g	0·6 g.	0·4 g.	0·2 g.
Total plasma protein g./100 c.c. plasma	6·83 g.	6·11 g.	5·52 g.	4·81 g.
Liver choline content mg./g. of fresh liver tissue	2·14 mg.	1·89 mg.	1·43 mg.	1·55 mg.

The total plasma protein concentration shows a definite decline as the daily intake of casein is reduced and the liver choline content also shows a similar fall.

Histology

On microscopical examination, the liver in animals which received 1·2 and 0·6 g. of casein per rat per day showed no pathological changes. Animals which received 0·4 and 0·2 g. of casein per rat per day, on the other hand, showed slight fatty change of the hepatic parenchyma around the central veins. But, in marked contrast to the animals in the low protein groups which were deprived of vitamins of B_2 complex, none of the animals in this group showed marked fatty change in the hepatic parenchyma around the central veins, accumulation of ceroid pigment or diffuse hepatic fibrosis.

3. Clinical Experiments

In addition to our experimental work, clinical treatment of decompensated portal cirrhosis cases with high protein, high caloric diets with vitamin supplements formed an important part of our studies on the 'cirrhosis of the liver' and this investigation was carried out in collaboration with Dr. N. J. Modi, M.B.B.S., M.R.C.P. (Lond.), in the medical unit of the Goculdas Tejpal Hospital, Bombay.

Twenty patients were treated with high protein diets which included skimmed milk powder, egg protein, fresh milk and casein hydrolysates. Vitamin supplements were given in the form of yeast tablets with injections of vitamin B_2 complex and liver extract in some cases. The majority of the patients come from the poorer classes and their dietary histories revealed gross deficiency in calories, protein and vitamin intake. Ten out of the twenty cases gave history of having had attacks of malaria. Nine cases were addicted to alcohol. Past

history of dysentery was present in four cases and jaundice in only two cases.

The presenting symptom of all patients was enlargement of abdomen, varying in duration from 15 days to two years. All the cases showed evidence of fluid in the abdomen. Spleen was enlarged in eight cases and liver was found enlarged in four cases only. The level of serum proteins was estimated before, during and after treatment as this afforded an objective evidence of the course of the disease. Total protein, albumin, globulin and euglobulin content of blood serum was estimated.

A common observation in all the patients before treatment was the fall in albumin and rise in globulin fraction of the serum proteins thus showing an altered albumin/globulin ratio from the normal. In the globulin fraction, the Euglobulin showed marked increase.

The period of observation during treatment was on an average 5 to 6 months. The results of therapy at the hospital have been quite encouraging. Patients treated with high protein diet showed definite clinical improvement. Ascites, edema and other manifestations of the disease disappeared in many instances. Clinical improvement was attended with a change in the serum protein level towards normal values.

A detailed account of these investigations will be shortly published elsewhere.

We wish to thank Major-General Sir Sahib Singh Sokhey, Kt., Director, Haffkine Institute, Bombay, for his constant encouragement and keen interest in this work.

1. Radhakrishna Rao, M. V., "Monograph on Cirrhosis of the Liver in Northern Circars—South India," *J. Ind. Med. Assoc.* (Dec. 1936; Jan.—July 1937). 2. Himsworth, H. P., and Glynn, L. E., *Clin. Sc.*, 1944, 5, 93-123 (1944).

Note.—The cost of printing this article has been met from a generous grant received from the National Institute of Sciences of India, New Delhi.

ORGANOLITES IN INDUSTRY

ADDRESSING the members of the Research Club, Kanpur, Dr. M. S. Bhatnagar said that Way first of all discovered the phenomenon of ion-exchange in inorganic substances like clay, silica, etc. Barrell gave the name of organolites to ion-exchanges got from synthetic resins. In 1935, Adama and Holmes working under G. T. Horgan, indicated the possibilities of using these synthetic resins for ion-exchange purposes. He prepared some cation and anion exchange resins from tannin barks and aromatic amines respectively. He found out that monohydric phenols cannot be used for the above purposes, but later on Sir Bhatnagar and his co-workers showed that ion-exchange resins can also be made from ordinary carbolic acid. These resins are of two types: (*a*) Cation exchange and (*b*) Anion exchange. Further, there are two types of cation exchangers—(*i*) having a strong acidic group like SO_3 and (*ii*) having a weak acidic group like carboxylic. The first one can be used for removing the cations from salts of both weak and strong acids while the second is useful only in removing the cations from salts of weak acids. These resins have as many applications as the types they can be made.

Continuing, Dr. Bhatnagar said that the cation exchangers are used for softening water and removing inorganic substances from sugar juice, apple and citrus fruit juice, dilute solutions of gelatin, etc. These juices are first passed through a cation exchanger which removes the metallic ion and later on, an anion exchanger removes the acid. Thus hard water can be very easily softened and also citric and lactic acids can be recovered. The most important contribution of these resins is in the purification of antibiotics like penicillin from moulds and in purification of alkaloids.