OBSERVATIONS ON THE PATHOGENESIS OF FIBROSIS IN RATS ON A PROTEIN DEFICIENT DIET

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IN biopsy specimens of the liver of patients with kwashiorkor, the presence of small collections of inflammatory and reticulo-endothelial cells surrounding degenerating liver cells has been noted. Although in uncomplicated and usually mild cases of kwashiorkor such as are seen in Jamaica, such cellular foci are not present in any quantity, yet they do occur regularly (Bras, 1955). They have been interpreted as representing cellular reaction to hepatocellular degeneration and/or necrosis. Following treatment, restitution is complete and there is no subsequent fibrosis (Back and Bras, unpublished).

We were struck by the presence of cellular foci similar to, but more abundant than, those which occur in the livers of patients with kwashiorkor during the examinations of the livers of many hundreds of rats, fed for various periods on one of the following experimental cirrhosis producing diets.

A.—Vitamin-free casein 8, lard (or Crisco) 38, cod liver oil 2, Sucrose 48, and U.S.P. salt-mixture 4.

B.—Vitamin-free casein 8, lard (or Crisco) 6, cod liver oil 2, Sucrose 80 and U.S.P., salt-mixture 4.

C.—Extracted peanut meal 30, casein 6, lard 18, cod liver oil 2, Sucrose 40 and U.S.P. salt-mixture 4.

D.—Extracted peanut meal 30, casein 6, Crisco 18, Sucrose 42, U.S.P. salt-mixture 4 and 3 drops perc. oil/wk.

E.—Extracted peanut meal 30, lard 18, cod liver oil 2, Sucrose 46 and U.S.P. salt-mixture 4.

The quantities in all the diets are in parts per hundred unless otherwise stated.

These cellular foci were noted mainly in those areas of the liver where fatty change had developed, *i.e.*, the non-portal regions (Fig. 1, 1*a*, 2) and almost exclusively in sites where fatty change existed, although this was not necessarily severe. They were more pronounced in the early stage of hepatic injury (50 days) than after fully developed general fibrosis.

This cellular infiltration was present (*a*) surrounding fatty liver cells, or, around globules of fat in disintegrating liver cells (Fig. 3, 4); (*b*) occasionally surrounding liver cells with eosinophilic degeneration of the cytoplasm, without apparent fatty change; (*c*) also in what appeared to be inter-cellular tissue, no liver cells being demonstrable in the areas involved (Fig. 5). This may mean,

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however, that this space had been occupied by liver cells which had disintegrated completely.

The foci of cellular infiltration were much less pronounced in the livers of animals fed the cirrhosis-producing diet with supplement of methionine, 50 mg. daily, which resulted in the deposit of not more than small amounts of fat in the liver cells. When 20 per cent of casein was present in the diet, no fatty change developed in the liver and foci of cellular infiltration did not occur.

Ceroid deposit was present in these areas of cellular infiltration, but in livers with little or no ceroid (Crisco as the source of fat in the diet) similar cellular foci occurred. Also, in well established dietary cirrhosis, the fibrous bands contained an abundance of ceroid but little or no cellular infiltrate. The presence of ceroid *per se* then cannot account for the cellular foci.

Apart from the well known fatty change, the liver cells showed such changes as mitoses, hyperchromatism and polyploidy. The portal triads sometimes displayed an increase in cells, especially around the bile ducts. Some of these cells were polymorphonuclears, but most of the cells were lymphocytes or reticulum cells and there was some proliferation of ductular lining epithelial cells. Liver cells, some well preserved, and others degenerating, necrotic, or containing fat globules, were enmeshed in the areas of fibrosis (Fig. 6).

Fatty cysts, as described by Hartroft (1950), were not much in evidence in our rats.

It appears, then, that in the livers of rats with this dietary deficiency changes other than fatty metamorphosis, occur which evoke cellular infiltrate. From the localization of the infiltrate, this additional factor seemed mostly to be localised around cells showing fatty change, but occurred occasionally away from the obviously fatty cells and was not always present in the precise centre of the lobules where the fatty change was most pronounced. In areas where the cellular foci were present, fibrosis developed, which coincided with the site of disappearance of liver cells.

It seems, that the factor which evokes the cellular infiltration is a slowly developing, piecemeal necrosis of the liver cells, frequently but not necessarily preceded by fatty metamorphosis. We believe, therefore, that parenchymal degeneration and cellular infiltration play a part in the development of cirrhosis due to dietary deficiency in rats, the fibrosis being the end result of a continuous gradual necrosis of the liver cells initially evidenced by cellular reaction surrounding these cells, and being either due to sustained fatty change or to the specific deficiency.

DISCUSSION

There is no doubt that degeneration and necrosis of liver cells have been observed by various investigators of cirrhosis. Some investigators of experimental dietary cirrhosis have incriminated the sustained fatty change in the liver cells as being responsible for the subsequent fibrosis (Daft, Sebrell and Lillie, 1942 ; Himsworth and Glyn, 1944).

Hartroft and Ridout (1951) considered the collapse of fatty cysts as the main cause of stromal condensation which results in the formation of fibrous septa in the liver of rats fed on a diet deficient in choline and methionine, but György and Goldblatt (1949) noted in the livers of such experimental animals the common

occurrence of degenerated or necrotic hepatic cells, in addition to the fatty change, preceding the fibrosis.

Koch-Weser *et al.* (1953) observed necrosis of liver cells, with accumulation of replacement cells, in rats on a high fat low protein diet. Similar foci were also seen by Nino-Herrera *et al.* (1954) in rats fed on a diet containing 9 per cent casein, with a choline supplement. Apparently the protein deficient diet induced cellular damage not prevented by a supplement of choline.

Popper and Schaffner (1957), in a study of human material (presumably the fatty livers of alcoholics) clearly described and gave pictures of fatty change, hepatocellular damage and necrosis, with the presence of reactive cellular infiltration.

So far as we are aware, most investigators, either implicitly or explicitly, have accepted the fact that sustained fatty change, perhaps supported by anoxemia in the central lobular areas (Himsworth, 1950), causes death of liver cells, while Hartroft (1950) demonstrated rupture and collapse of hepatic fatty cysts. Fatty cysts did occur in our material, but they were not striking, and their collapse did not seem to account in any great part for the development of the fibrous septa. Differences in the diet employed by Hartroft and by us may account for this.

We are impressed by the apparent importance of degeneration and necrosis of liver cells and the cellular reaction they evoke, both in experimental dietary cirrhosis and in human cases of protein deficiency. We incline, therefore, to the view that such degeneration and the inflammatory infiltrate are important pathogenetic factors in the development of fibrosing liver disease. They apparently may be caused by choline and methionine (protein) deficiency, while toxic or infectious noxious agents may enhance their development and increase their number. When the cellular foci are present in sufficient quantity they are indicative of liver disease progressing toward fibrosis.

SUMMARY

White, male, Sprague-Dawley rats on a protein deficient diet developed fatty change in parenchymal cells of the liver, followed by fibrosis. Under these circumstances, however, the fibrosis seemed to occur as a result of a continuous gradual necrosis of liver cells rather than through formation of fatty cysts.

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EXPLANATION OF PLATES.

FIG. 1.—Low power view of liver lobule with portal triad (P) and central vein (C.V.). Note cellular foci towards the lobular centre. H.E. Section ×125.

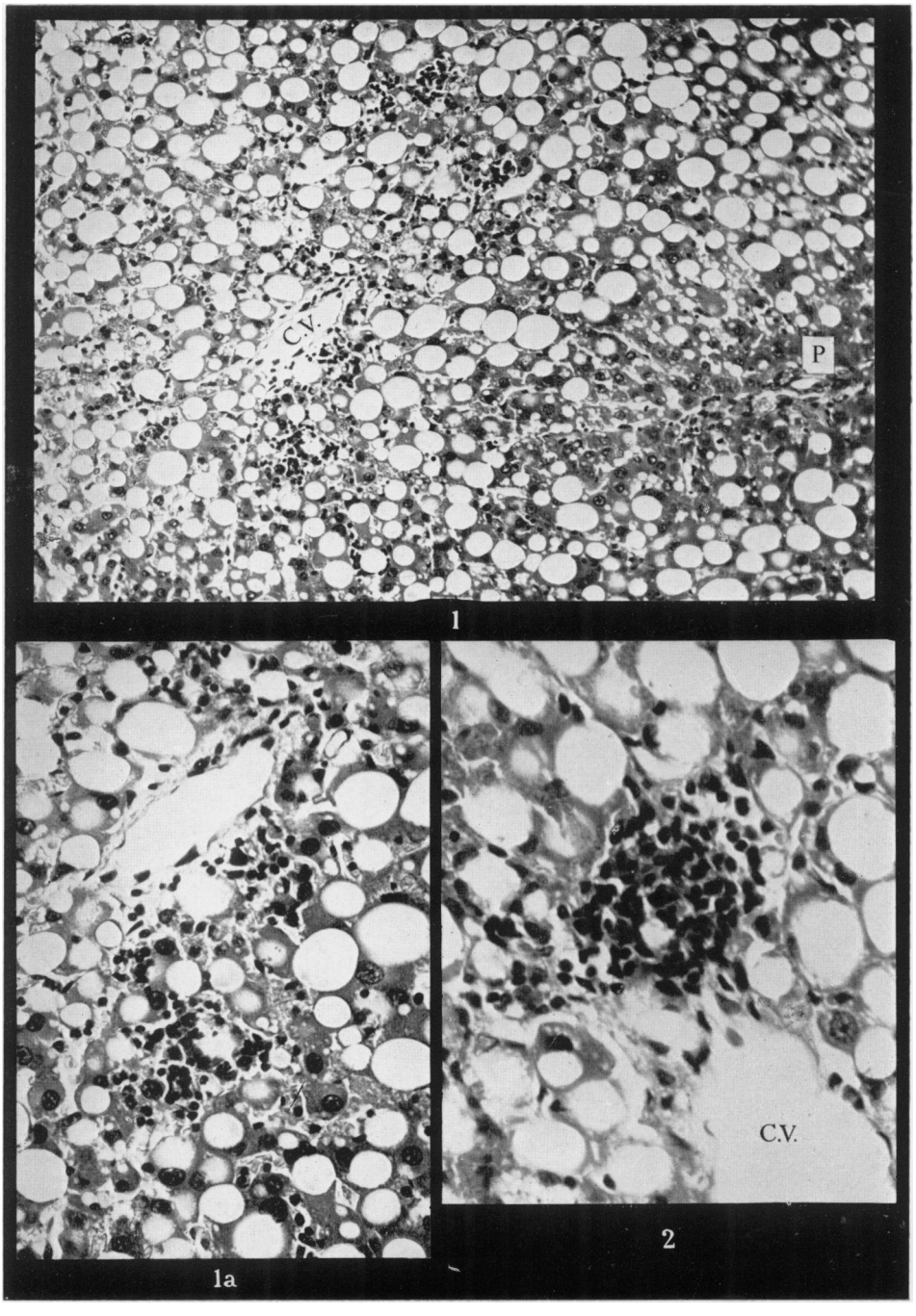
FIG. 1A.—Close-up of centrolobular area in Fig. 1. ×243.

FIG. 2.—Focus near central vein (C.V.). H.E. ×300.

FIGS. 3 and 4.—Focus surrounding fatty liver cell (fat globules). H.E. ×427.

FIG. 5.—Focus located " interstitially ". H.E. ×427.

FIG. 6.—Centrolobular area showing fibrosis. H.E. ×127.



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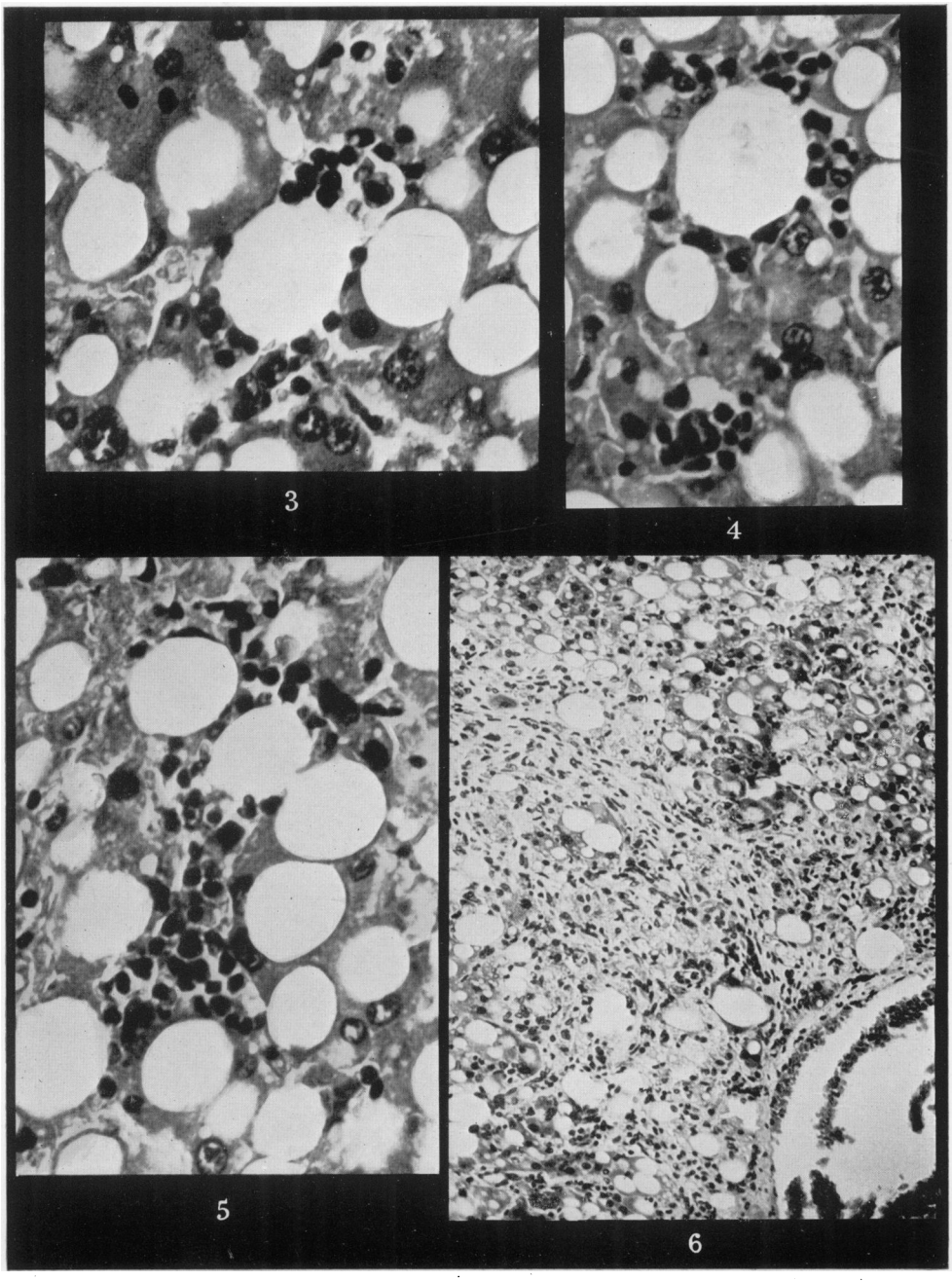

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