# VENO-OCCLUSIVE LESIONS IN LIVERS OF RATS FED *CROTALARIA FULVA*

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WHEN cirrhosis of the liver in Jamaican infants was first described by McFarlane and Branday in 1945 a suggestion was made that the disease might be due to the toxic action of herbs, used by the native population (so called bush-teas). The underlying liver lesion (*i.e.* occlusion of the centrilobular hepatic vein and subsequent centrilobular fibrosis) was not recognised until 1954 (Bras, Jelliffe and Stuart) and the illness was then named veno-occlusive disease of the liver (VOD). Fibrosis of this type may lead to cirrhosis of the liver and it is estimated that in Jamaica VOD is responsible for 30 per cent of cirrhosis found at autopsy (Bras, Brooks and Watler, 1961).

Animals known to have died following the ingestion of ragwort (Senecio species), also have occlusive lesions apparently comparable to human VOD (Bras, Berry and György, 1957 ; Bras *et al.*, 1954). Senecio, however, is only rarely being used as a bush-tea in Jamaica.

A study of VOD patients in Barbados definitely incriminated another plant, *Crotalaria retusa* (Stuart and Bras, 1956). When this lead was followed in Jamaica it was learned that *Crotalaria fulva* was a well recognised bush-tea. Crotalaria and Senecio both contain pyrrolizidine alkaloids known to be liver toxins (Schoental, Regina and Magee, 1959).

For several years efforts have been made in our laboratory to reproduce VOD in animals by the oral administration of *Crotalaria fulva* infusions, *i.e.* approximating the conditions believed to lead to the disease in the Jamaican population. These experiments were successful in cows (Bras *et al.*, 1957) and in goats (Lindo and Bras, unpublished), but in rats positive results have been obtained rarely and inconstantly (Hill, Stephenson and Filshie, 1958, reported occlusive lesions in rats but following intra-peritoneal injections).

A different experimental design, described in this paper, has now enabled us to produce VOD in rats with regularity. Identical results were obtained in both laboratories, Jamaica and Philadelphia.

The histological events leading up to the appearance of the occlusive lesion are described. Abnormalities of serum and liver transaminase levels and of liver glycogen and water content are also described and their relevance to a proposed pathogenesis of the lesion is discussed.

## MATERIALS AND METHODS

*Animals.*—Wistar rats of both sexes, of weights 70–170 g. were used. They were kept in individual cages at room temperature (25–33°) and fed a Purina rat chow and water *ad lib.* A water extract of *Crotalaria fulva* was prepared as described elsewhere (Stirling and Urquhart, 1962) and administered by stomach tube. Doses of 0·8–1·5 g. dry wt./kg. rat

body weight were given, depending upon the toxic activity of the extract. Consecutive samples of blood were obtained by incision of a tail vein. Animals were killed by transection of the cervical spine after light ether anaesthesia and exsanguination.

*Histology.*—Tissues were fixed in 10 per cent buffered formalin and embedded in paraffin. Sections were stained with haematoxylin and eosin, periodic acid-Schiff, and Masson's trichrome (MT).

*Biochemistry.*—Glutamic pyruvic transaminase (GPT) in serum and liver was determined by the method of King scaled down by a factor of 4 for liver and 8 for serum. The serum values were reproducible $\pm 10$ per cent for a 25 $\mu$l. sample.

Liver glycogen was determined by the method of Kemp and Kits van Heiningen (1954).

## RESULTS

In humans the earliest lesions observed have been occlusion of branches of the hepatic veins with concomitant severe centrilobular congestion and loss of parenchymal cells in the centrilobular area. At this stage severe ascites is present clinically. Subsequently, collateral circulation relieves the congestion and ascites disappears; Fig. 1 shows this situation in the liver of a child with subacute VOD. Fig. 2 shows an identical lesion from the liver of a rat killed 9 days after a single oral dose of *Crotalaria fulva*. Figs. 3 and 4 show respectively non-portal fibrosis in the livers of a child with chronic VOD and of a rat killed 12 days after a single oral dose of Crotalaria. Again, the lesion in the animal closely mimics that in the child.

Lesions identical to those in human VOD appear in rats 8–12 days after a single oral dose of Crotalaria extract. The dose will, therefore, have to be chosen with some care. Lesions are not present when larger doses cause the animals to die earlier, while smaller doses of the extract may not be lethal. Again, repeated small oral doses do not give rise to such occlusive lesions but to a cirrhosis mainly portal in type (György, Goldblatt and Bras, unpublished). Different investigators may thus produce and report very different liver lesions, according to the experimental design.

Individual susceptibility in the rats varies very widely as is to be expected. In rats receiving a dose of this order (0·8–1·5 g. dry wt./kg. body wt.), deaths are not normally distributed against time but occur in two peaks, one at 2–3 and one at 6–7 days.

Fig. 5 shows that approximately one-third of the animals die in each of these peaks, the remainder usually succumbing within 25 days.

At 24 hr. these animals begin to look ill, crouching in a corner with their fur standing up. At this time they have a swollen belly due to a stomach enormously distended with food. A pilot experiment suggests that these animals have gained more weight in the first 24 hr than controls. Since this gain is not due to ascites, it seems that the distended stomach is connected with increased appetite and not only with pyloric spasm. Subsequently, they lose their appetite and become apathetic and dirty. The less severely affected ones may become alert and clean again at 4–5 days and relapse at 6–7 days.

At necropsy, naked eye changes in the liver appear first at 24–48 hr. when a diffuse red speckling accentuates the lobular pattern. At 48 hr. the majority have ascites, sometimes bloody. By 3 days all have a large dark liver which is firm to the touch and granular on the surface and in section. From this time on it is strikingly evident both in handling the liver and in the preparation of homogenates that a large amount of blood is trapped in the liver tissue.

Dark patches occur frequently in the lungs at all times. Pleural effusion often accompanies the ascites and is sometimes present alone.

*Histology*

Pyrrolizidine alkaloids cause a severe centrilobular necrosis and the early stages of Crotalaria poisoning follow this pattern. The earliest histological change, as also demonstrated by Stirling and Urquhart (1962), is a centrilobular loss of glycogen. This may appear as early as 3 hr. and is present in all animals

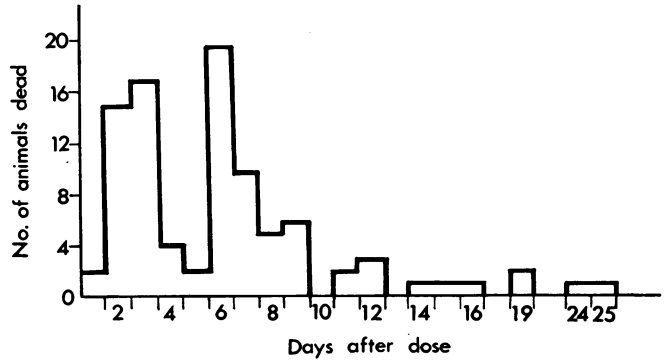



FIG. 5.—Distribution of deaths against time. Ninety-three rats receiving a single oral dose of Crotalaria extract 0·8–1·5 g. dry weight/kg. body weight.

at 12 hr. At 24 hr. centrilobular congestion is visible in the H. and E. stain, while at 2 days centrilobular necrosis is obvious. The severity varies very greatly. Necrosis may be confluent in one part of a lobe and involving one-third or less of the lobule in other parts. In general, the median and left lateral lobes are affected earliest and most severely, and the caudate lobes latest and least. At 3 and 4 days large amounts of blood appear among the necrotic tissue. At first this is evenly distributed among the dead cells. Later the cells may disappear giving rise to " lakes " of blood (Fig. 6). A similar lesion was described as " cysts " by Selzer and Parker in experimental retrorsine poisoning in rats (1951). Sometimes the blood appears in a ring between the healthy and the necrotic tissue. At this time many central veins appear distorted, sometimes as a flat ellipse, sometimes indented in more than one place to produce a triangular appearance. They look as if they had been squashed. When the " lakes "

EXPLANATION OF PLATE

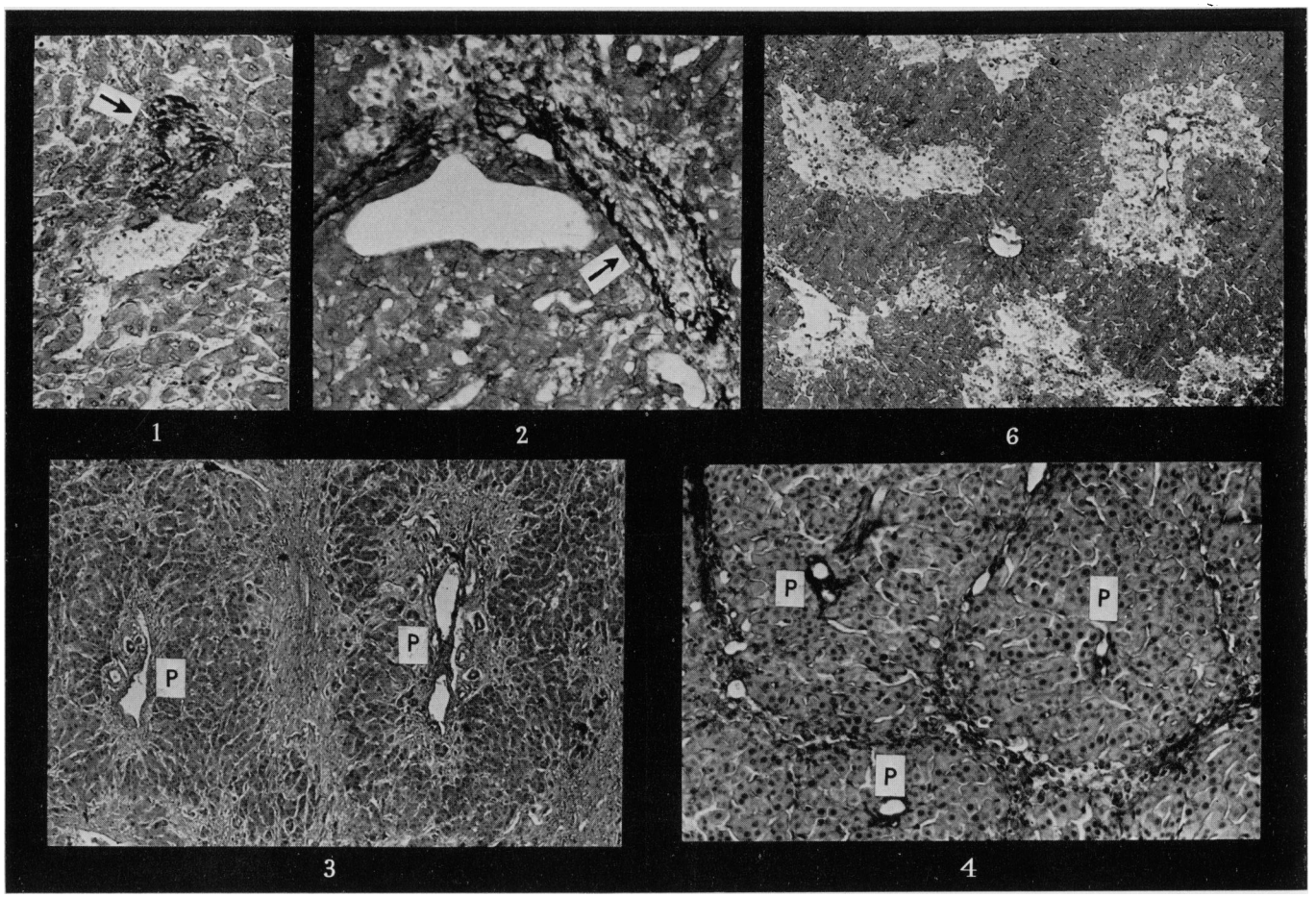
FIG. 1.—Human VOD. Occlusion of centrilobular vein (arrow) with adjacent collateral channels. Mallory's trichrome stain ×125.

FIG. 2.—Occlusive lesion from liver of rat killed 9 days after a single dose of Crotalaria. Arrow indicates occluded vein. Clear spaces are collateral channels. Mallory's trichrome stain ×125.

FIG. 3.—Human VOD. Non-portal fibrosis, leaving portal areas (P) relatively unaffected. Fast green connective tissue stain ×50.

FIG. 4.—Early non-portal fibrosis (portal triads marked P) from liver of rat killed 12 days after single oral dose of Crotalaria. Mallory's trichrome stain ×125.

FIG. 6.—Centrilobular lakes surrounding distorted central veins from liver of rat killed 3 days after a single oral dose of Crotalaria. Mallory's trichrome, ×50.




McLean, Bras and György.

are very pronounced, distorted central veins may be seen lying in them (Fig. 6). In other cases blood is frequently seen to lie touching, but outside, a distorted vein. From this time on the parenchymal tissue may show either a gradual recovery with regeneration of liver cells filling up the vacant centre of the lobule, or progressive necrosis and engorgement with blood, leaving a rim of surviving cells around the portal tracts only 1 or 2 cells thick. These changes are also very unevenly distributed, within the same liver. In either case, the central veins gradually fill up with collagen fibres staining blue in MT while collateral channels (Fig. 2) develop. A single layer of hypertrophic endothelial cells may be seen at 3–4 days, but they do not proliferate to fill up the lumen. The block in the central vein may appear first as a thickening of the endothelium or as a fine network right across the vein. These changes appear after about 7 days. They progress between 8 and 12 days to a more or less solid block in which the outline of the original vein is still visible (Fig. 2). Concurrently collateral channels develop in the vicinity. The scar of the old vein, remarkably enough, seems to disappear and the new channels are eventually distinguishable from normal central veins only by being more numerous.

*Biochemistry*

Fig. 7 shows the distribution against time of serum glutamic pyruvic transaminase (SGPT) in rats dosed as above. From an upper limit of normal of 280

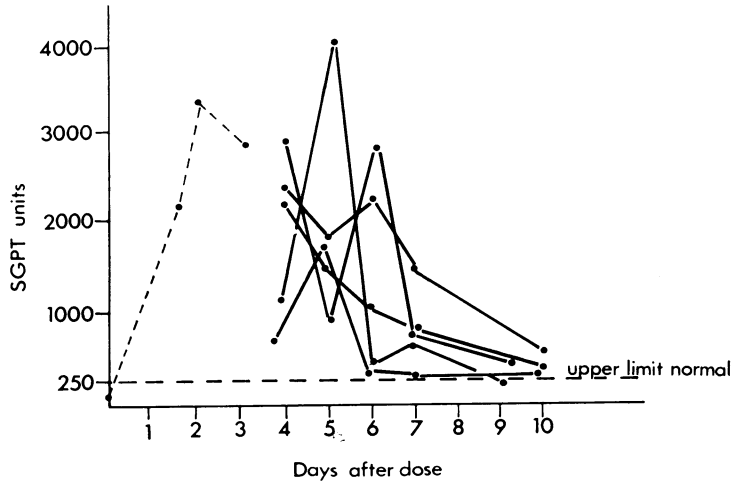



FIG. 7.—SGPT levels in rats 0 to 10 days after a single dose of Crotalaria. Values for 0, 1½, 2 and 3 days are means of 15 animals. Values for 4, 5, 6, 7, 9 and 10 days are consecutive readings on 5 individual animals. Units = μmoles substrate converted/ml. serum/hr.

units (μmoles converted/100 ml. serum/hr.) the level rises to a peak at 2–3 days. This rise is evident in all animals. The further course cannot usefully be plotted as a mean because of the extreme variation in susceptibility of the animals. Individual plots for animals surviving to 5 days or longer show that most have a second sharp peak at 5 or 6 days. For the sake of clarity only 5 individual animals

have been plotted here. Similar patterns have been shown in more than 25 animals.

Some further measurements were made on similar rats killed at daily intervals to 10 days (Table). Fat free dry weight (FFD) remained constant. Water, on a basis of FFD, was already rising at 1 day, reached a peak at 7–9 days and had returned to normal in about half the animals at 10 days. Total liver transaminase reflects the serum transaminase findings mentioned above. A sharp fall at 1 day is followed by a much more gradual fall to 5 days. A second sharp fall at 6 days leads on to gradual recovery, about half the animals showing normal values at 10 days. While one cannot, in the absence of any information about clearance of serum transaminase, draw a firm conclusion about the connection between serum and liver transaminase values, it is tempting to accept these figures at their face value and to say that liver transaminase falls sharply as a result of sharp rises in serum transaminase. Total liver glycogen follows a similar two-phased pattern. It falls sharply at 2 and 3 days, recovers slightly at 4 and falls again at 5 and 6. Chemical determinations have not been made at 9 and 10 days. Histological observations suggest that the glycogen content of parenchymal cells is normal in some animals at this time. Since the liver contains an abnormally large amount of fibrous tissue at this time, glycogen values on a basis of FFD would probably still be below normal.

TABLE.—*Changes in Liver Composition Following a Single Oral Dose of Crotalaria Extract*

| | Values in control animals | Days following Crotalaria administration | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 9 | 10 |
| Fat free dry weight (FFD) (g. in total liver per 100 g. body weight) | 1·3 | 1·2 | 1·3 | 1·4 | 1·2 | 1·1 | 1·2 | 1·3 | — | — |
| Total liver GPT (Megaunits) | 15·4 | 10·6 | 12·3 | 9·8 | 9·2 | 9·0 | 3·8 | 6·3 | 12·4 | 13·4 |
| Total liver water (kg. per kg. FFD) | 2·5 | 2·9 | 3·0 | 3·5 | 3·5 | 4·1 | 4·0 | 4·4 | 4·5 | 3·8 |
| Total liver glycogen (mg. per 100 g. body weight) | 137 | 127 | 57 | 38 | 64 | 22 | 17 | 30 | — | — |
| Number of animals | 7 | 5 | 5 | 8 | 5 | 8 | 5 | 10 | 5 | 5 |

## DISCUSSION

Four points made above appear to require explanation: (1) Ascites appears at 2 days, histological venous occlusion at 8. (2) The serum and liver glutamic pyruvic transaminase levels and the liver glycogen levels point to two waves of necrosis, one at 3 and one at 6 days. (3) At 3 and 4 days the liver appears to be full of blood some of which is lying next to distorted, but histologically patent vessels, apparently unable to enter these vessels and drain away. (4) From 3 days the texture of the liver is quite abnormal, being firm to the touch.

The firmness is not due to fibrosis. It is tempting to attribute it to the presence of large quantities of blood. Since normal rat livers offer effectively no resistance to outflow from the sinusoidal bed (Brauer, 1963), blood can be trapped in the liver in quantities sufficient to render it firm only in the presence of some degree of obstruction of hepatic outflow. If one proposes a functional block to the outflow of blood (such as might arise from collapse of part of the hepatic venous tree) present from 2 days, one can explain the above points. Ascites follows obstruction of outflow from the liver.

The first rise of SGPT we ascribe to the hepatotoxic alkaloid damaging hepatic cells, the second to further necrosis following centrilobular stagnation attendant on the functional block to blood flow. The presence of lakes of blood in sections containing histologically patent central veins indicates an interruption of the blood pathway which normally connects the liver tissue with the hepatic vein. The exact site of this interruption at this stage is a matter for conjecture, a possible explanation being that veins which appear patent in histological preparation could be collapsed in the living animal.

Collapse of parts of the hepatic venous tree is not a fanciful proposition. Brauer has shown that the most vulnerable portion of the hepatic venous tree in the rat can be collapsed by small external pressures on the liver (Brauer, 1963) and that it does suffer rhythmical collapse with the excursions of the diaphragm. In these circumstances the Crotalaria alkaloids could act to cause collapse either directly in the vein, or indirectly by some relatively small, but continuous, increase of pressure on the liver. Such actions appear to be peculiar to the Crotalaria and Senecio alkaloids. Since Hill, *et al.* (1958) have produced occlusive lesions with monocrotaline there is at present no need to postulate that the " occlusive action " is due to some other constituent of the " tea ".

SUMMARY

Veno-occlusive lesions, comparable with those seen in humans have been produced in the livers of rats fed *Crotalaria fulva*. To produce veno-occlusion the dose had to be chosen so that the rats die approximately between 8 to 12 days following a single dose.

Evidence is presented, from necropsy findings, from the histology, and from the serum glutamic pyruvic transaminase pattern, that histological occlusion of the central veins is preceded for several days by a functional block to blood flow.

The possible role of central vein collapse in the production of the functional block is discussed.

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