THE TOXIC EFFECT OF CROTALARIA EXTRACT ON THE LIVER OF RATS

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INFUSIONS of plants used as " bush teas " or herbal remedies in Jamaica are believed to be concerned in the aetiology of veno-occlusive disease of the liver. This disease, as described by Bras and Hill (1956), is characterised by ascites, hepatomegaly and occluded hepatic veins. The plants used include the genus Crotalaria which contains the pyrrolizidine group of alkaloids usually referred to as the " senecio alkaloids ". The pathogenesis of poisoning by these alkaloids is undetermined, and there is no agreement as to whether the primary lesion is in the vascular system or the liver cells. In this work the acute liver changes, culminating in centrilobular necrosis, were studied in rats given a single dose of crotalaria extract.

METHODS AND MATERIALS

The extract was prepared by boiling for 1 hr. the fresh leaves of the plant *Crotalaria fulva*. The extract was concentrated to one-fourth of its original volume by steam distillation and administered in this form by stomach tube.

The dry weight of the extract was estimated and the dose given was based on this. It was found that 4 mg. of the extract per gram body weight was sufficient to produce liver damage in about 24 hr. in most rats, regardless of sex or age. This dosage was used throughout.

Stock albino rats of either sex weighing 40–180 g. were used. They were fed on " Purina " rat chow and given as much water as they wanted. Thirty-six rats divided into 6 batches were given crotalaria extract and killed at intervals ranging from 3–32 hr. by a sharp stunning blow on the head. Two of the batches of rats were starved 3 hr. before the extract was given. The 36 control animals were not given extract, but their treatment differed in no other way from the poisoned rats.

Blocks of liver tissue were fixed in 10 per cent neutral buffered formaldehyde and paraffin sections were cut and stained by : haematoxylin and eosin ; Altmann's aniline acid fuchsin and Mallory's phosphotungstic acid haematoxylin for mitochondria ; the periodic acid-Schiff method for glycogen, and by the Feulgen technique. Frozen sections were cut and stained with Sudan IV.

Other blocks of tissue were frozen in dry ice and butyl alcohol and cut in a cryostat. Some of the sections were treated with *p*-nitrophenyl substituted ditetrazole (Nitro B.T.) to demonstrate succinic dehydrogenase activity according to the method of Nachlas, Tsou, de Souza, Chang and Seligman (1957).

Other sections were fixed in a mixture of equal parts of ethyl-alcohol and diethyl-ether for 30 min., hydrated by passage through graded alcohols and stained with acridine orange for ribonucleic acid. These sections were examined under a fluorescent microscope.

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RESULTS

Macroscopic appearances

With the exception of a few petechial haemorrhages in other organs, the lesions were restricted to the liver, which showed an increasing degree of congestion until 18 to 24 hr. after the administration of the extract when necrosis became visible as small yellow areas scattered in a regular manner throughout the substance of the liver.

Microscopic appearances

Within 3 hr. of the administration of the crotalaria extract, PAS positive material (presumably glycogen) had disappeared from the centrilobular areas. In the starved rats it failed to reappear in the centrilobular areas. A reduction of succinic dehydrogenase activity was noted in these same areas but this was not marked. The only change in the haematoxylin and eosin preparations was separation of the cords of liver cells.

At 6 hr. the only additional change was centrilobular congestion.

At 9 hr. the cells in the centrilobular areas stained deeply with eosin and small droplets of fat were distributed evenly throughout their cytoplasm. Diminution of succinic dehydrogenase activity was now marked and swollen mitochondria were seen in a few cells, particularly those adjacent to the centrilobular veins. In the periportal part of the lobules there was marked enlargement of many nuclei.

At 12 hr. the fatty change in the centrilobular areas was severe and fine droplets of fat were present in the cells of the peripheral zones. Ribonucleic acid was diminished in the centrilobular zone. The mitochondria of the cells around the centrilobular veins were agglutinated and some of the cells showed intracytoplasmic bodies which appeared to increase in size until they were extruded into the sinusoids. In the central parts of the lobule the nuclei showed a migration of chromatin towards the nuclear membrane, and a loss of the nucleoli.

From 18 hr. onwards there was necrosis and disappearance of centrilobular cells. Surviving centrilobular cells were either shrunken, or ballooned with what appeared to be plasma. Eventually the centrilobular areas consisted of little more than lakes of blood.

DISCUSSION

There is no agreement as to whether the primary lesion in senecio poisoning is in the vascular system or liver cells. Davidson (1935) as well as Rosenfeld and Beath (1945) considered a vascular lesion to be the essential one. Davidson thought the larger vessels of the liver were concerned but Rosenfeld and Beath reported capillary injury not limited to the liver, but most prominent there. Chen, Harris and Schulze (1940), like Harris and his colleagues (1942) emphasised the cellular

EXPLANATION OF PLATES

FIG. 1.—Disappearance of glycogen from centrilobular zones. PAS ×50.

FIG. 2.—Diminution of succinic dehydrogenase activity in centrilobular zones. Nitro B.T. ×40.

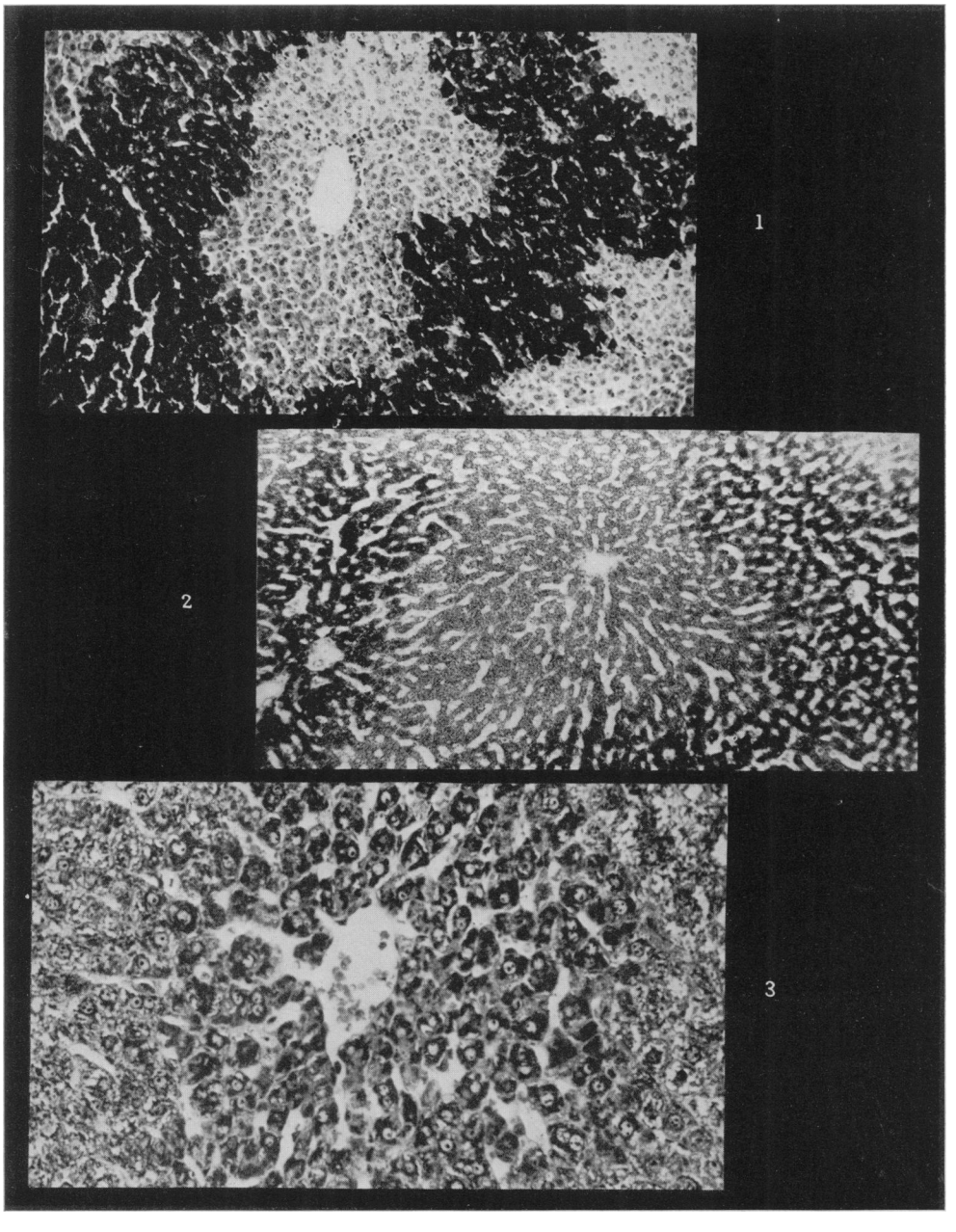
FIG. 3.—Agglutination of mitochondria in a centrilobular zone. Mallory ×125.

Fig. 4.—Loss of ribonucleic acid from a centrilobular zone. (Centrilobular vein on the right and portal tract on the left.)

FIG. 5.—Fatty change in a centrilobular zone. Sudan IV ×125.

FIG. 6.—Intracytoplasmic bodies. H. and E. ×500.

FIG. 7.—Migration of chromatin and nucleoli to the nuclear membrane. Feulgen ×500.



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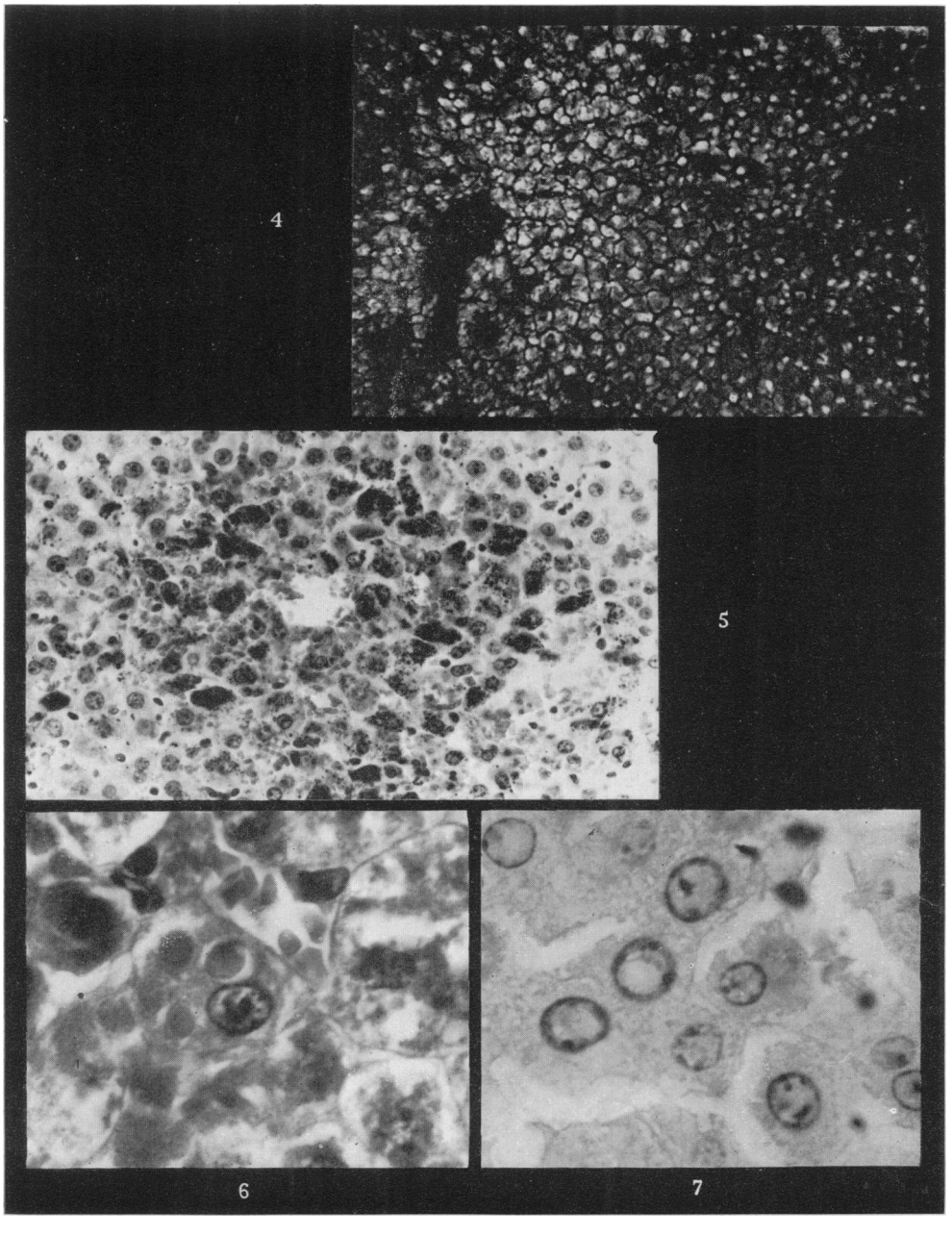

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necrosis, while Selzer, Parker and Sapeika (1951) thought a toxic effect on both the liver cells and the vessels was concerned. They described cellular proliferations in the branches of the hepatic veins which appeared to be capable of blocking the vessels and being converted to fibrous tissue. Occlusions of the hepatic veins in rats given intraperitoneal injections of monocrotaline, one of the pyrrolizidine alkaloids, have been reported by Hill, Stephenson and Filshie (1958). These occlusions were similar to those in veno-occlusive disease as described by Bras, Jelliffe and Stuart (1954) in human material. Bull, Dick and McKenzie (1958) reported a primary toxic action in the liver cell. Schoental (1959) thought the thickened centrilobular veins with narrowed lumina which she observed were a sequel to haemorrhage and stagnation of blood.

In our material the demonstration of a constant sequence of cellular changes preceding centrilobular necrosis suggested that the toxic action of crotalaria extract was exerted directly on the liver cells. No occlusions of the hepatic veins were noted, and the only constant vascular changes encountered were congestion followed by " laking " of the blood in the centrilobular zones. However any conclusion drawn from our experiment must be applied with caution to human veno-occlusive disease. Not only because of anatomical differences between the hepatic veins in man and the rat, but because our crotalaria extract was given in a single dose.

SUMMARY

Crotalaria extract similar to that used as a bush tea in Jamaica has a primary toxic action on the rat liver cell when given in a single dose. No vascular occlusive lesion was noted.

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REFERENCES

Bras, G. and Hill, K. R.—(1956) *Lancet*, ii, 161.
Idem, Jelliffe, D. B. and Stuart, K. L.—(1954) *Docum. Med. Geog. Trop.*, **6**, 43.
Bull, L. B., Dick, A. T. and McKenzie, J. S.—(1958) *J. Path. Bact.*, **75**, 17.
Chen, K. K., Harris, P. N. and Schulze, H. A.—(1940) *J. Pharmacol.*, **68**, 123.
Davidson, J.—(1935) *J. Path. Bact.*, **40**, 285.
Harris, P. N., Anderson, R. G. and Chen, K. K.—(1942) *J. Pharmacol.*, **75**, 69.
Hill, K. R., Stephenson, G. F. and Filshie, I.—(1958) *Lancet*, *i*, 623.
Nachlas, M. M., Tsou, K. C., de Souza, E., Chang, C. S. and Seligman, A. M.—(1957) *J. Histochem. Cytochem.*, **5**, 420.
Rosenfeld, I. and Beath, O. A.—(1945) *Amer. J. clin. Path.*, **15**, 407.
Schoental, Regina—(1959) *J. Path. Bact.*, **77**, 485.
Selzer, G., Parker, R. C. F. and Sapeika, N.—(1951) *Brit. J. exp. Path.*, **32**, 14.