AN EXPERIMENTAL STUDY OF SENECIO POISONING IN RATS.

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IN another paper the findings in twelve cases of senecio poisoning in man are described (Selzer and Parker, 1951). Autopsies performed on the six fatal cases revealed ascites and a striking centrilobular haemorrhagic lesion in the liver which was shown to be due to occlusion of the hepatic veins (Chiari's syndrome). As the experimental production of Chiari's syndrome had not been reported by previous workers on senecio poisoning (Cushny, 1910 ; Theiler, 1919 ; de Kock, du Toit and Steyn, 1931 ; Davidson, 1935 ; Harris, Anderson and Chen, 1942), we decided to study afresh the lesions produced in rats by the senecio plant and its alkaloids, with special reference to any possible changes in the blood vessels of the liver. As our human cases were all persons in poor circumstances, some of our animals were placed on a low protein diet which was known to be effective in the production of massive hepatic necrosis.

METHODS.

In all experiments albino rats weighing from 60 to 120 g. were used.

Two diets were employed. The first was a normal stock cubed diet consisting of yellow maize meal 48 per cent, peanut meal 10 per cent, meat meal 15 per cent, fish meal 2·5 per cent, oatmeal 15 per cent, barley 7·5 per cent, lucerne 1 per cent, salts 0·5 per cent, and lime stone powder 0·5 per cent, which was supplemented by green vegetables twice a week. The second diet was a low-protein diet suggested by Gillman (1948, personal communication), and consisted of 90 per cent potato starch and 10 per cent food yeast, being prepared as follows : To 500 ml. of boiling water were added 90 g. of potato starch previously made into a smooth paste with 100 ml. of water. The mixture was stirred thoroughly and allowed to boil for 2 minutes. When cool, 10 g. of food yeast in 20 ml. of water were added and mixed in very thoroughly. This diet was administered to rats *ad libitum*, and was not supplemented by vitamins, green vegetables or salt mixtures.

The most suitable available senecio alkaloid was retrorsine, which is present in *Senecio ilicifolius*, although not the most plentiful alkaloid in that plant. It is present also in other senecio species. The alkaloid was supplied by Dr.

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H. L. de Waal, of Pretoria. Solutions were prepared by dissolving a weighed quantity of the alkaloid in an equimolecular amount of hydrochloric acid. This was administered by stomach tube, except in one experiment in which it was injected subcutaneously. In another experiment alcoholic and aqueous extracts of the plant, *Senecio ilicifolius*, were given by mouth.

The animals which did not die were killed by carbon monoxide gas in Experiment 1, and by dislocation of the neck in Experiments 2 to 6. Tissues were fixed in 10 per cent formalin, and paraffin sections were stained with haematoxylin and eosin and with van Gieson's stain. Frozen sections stained with scarlet red were examined for fat.

RESULTS.

Experiment 1 : Retrorsine hydrochloride 1 mg. per 10 g. body weight was administered in a single dose by mouth to 30 rats on a normal diet, and to 30 rats on a low protein diet. Of the 30 rats on a normal diet, 4 were dead by the third day, 8 died subsequently, and the survivors were killed on the twelfth day. In all the rats that died and in two of those killed on the twelfth day there was a haemorrhagic zonal necrosis of the liver. In the remainder the liver appeared normal.

Of the 30 rats which had been on a low protein diet for 21 days prior to the administration of retrorsine, 25 were dead 3 days after receiving the alkaloid, and 5 died within the next four days. Typical haemorrhagic zonal necrosis was found in 27 of the 30 rats. The low protein diet therefore appeared to enhance markedly the effect of the alkaloid.

Twenty control rats fed on a normal diet and housed under identical conditions did not develop any hepatic lesions. A second control group of 21 rats were fed a diet of food yeast and potato starch and did not receive retrorsine. Three died before the 35th day of the experiment, and these did not show any liver lesions. The remaining 18 rats died between the 35th and 70th days, and all showed a necrosis which was massive and not zonal in type.

At autopsy it was found that retrorsine produced similar hepatic lesions, both in the rats on a normal diet and in those on a low protein diet. Macroscopically the liver was tense and firm, with a uniform mottled appearance resembling that of passive congestion. Nine rats showed haemorrhage into the lungs, varying in size from minute petechiae to lesions involving more than half a lobe. Four of the animals had frank haemorrhage into the peritoneal cavity, the source of which could not be ascertained. In none was there marked oedema of the large bowel such as was seen in the human cases.

Histologically the liver damage was often more marked than the macrosopic appearance suggested. In all cases the lesion was essentially zonal, being most marked around the central vein, but frequently involving two-thirds of the liver lobule (Fig. 1). In the affected zones most of the liver cells had disappeared, and those that remained were necrotic. The same areas were filled with red blood corpuscles, so that the appearance suggested a recent haemorrhage. The picture contrasted markedly with that seen in the control rats fed a diet of food yeast and potato starch. The latter group of animals showed large areas of massive hepatic necrosis scattered throughout all the lobes, and separated by liver tissue which was normal or showed fatty change only. Histologically the appearance of massive necrosis was confirmed. Much of the necrotic tissue

was still *in situ*, although in some regions the dead cells had disappeared and the sinusoids were dilated.

Experiment 2: In an attempt to trace the evolution of the lesion produced by the senecio alkaloid, 43 rats on a normal diet were given retrorsine hydrochloride 1·5 mg. per 10 g. body weight in a single dose by mouth. Animals were killed at each three-hourly interval up to the 30th hour, and thereafter at frequent but irregular intervals up to the 72nd hour. Considerable variations were observed in the time of onset of the earliest demonstrable lesion and in the extent of the fully developed picture. Thus, whilst in some rats killed after 6 hours early lesions were seen, others killed 15 hours later showed a normal liver. Detailed results are recorded in Table I.

TABLE I.—*Experiment* 2.

Time in hours.	Rat.	Histological findings.
3	1 and 2	No abnormality seen.
6	3 and 4	No abnormality seen.
	5	Periportal sinusoidal dilatation.
	6	Centrilobular sinusoidal dilatation. Early degeneration of liver cells.
9	7	No abnormality seen.
	8	No abnormality seen except eosinophilic infiltration of portal tracts.
12	9, 10 and 11	No abnormality seen.
	12	Centrilobular haemorrhagic necrosis.
	13	Centrilobular haemorrhagic necrosis.
15	14	Slight centrilobular haemorrhagic necrosis.
18	15 and 16	Centrilobular sinusoidal dilatation. Infiltration with polymorphs.
21	17 and 18	No abnormality seen.
	19 and 20	Centrilobular haemorrhagic necrosis with disappearance of many liver cells. Infiltration with polymorphs.
24	21	Very extensive centrilobular haemorrhagic necrosis.
	22	Very slight centrilobular necrosis.
27	23 and 24	Occasional necrotic cells in central zones only.
30	25 and 26	Extensive centrilobular haemorrhagic necrosis. Heavy infiltration with polymorphs.
	27 and 28	No abnormality seen.
36	29 and 30	Extensive centrilobular haemorrhagic necrosis. Heavy infiltration with polymorphs.
	31 and 32	Extensive centrilobular haemorrhagic necrosis. Liver cells largely disappeared in central zones, which contain many macrophages. Slight "endothelial swelling."
48	33 and 34	Centrilobular "cysts." Slight "endothelial swelling."
52	35 and 36	Centrilobular "cysts" containing a few macrophages and polymorphs.
55	37	Centrilobular "cysts" containing macrophages and polymorphs. Slight "endothelial swelling."
60	38 and 39	Centrilobular "cysts" containing macrophages and polymorphs. Slight "endothelial swelling."
72	40 and 41	Centrilobular "cysts" containing macrophages. Slight "endothelial swelling."
	42 and 43	Slight centrilobular necrosis and "endothelial swelling."

The earliest change was seen at 6 hours. In two of four rats killed at this stage the liver appeared normal. The others showed dilatation of sinusoids, which was centrilobular in one rat and periportal in the other. In the former there was evidence of early degeneration of liver cells.

Two of five rats killed 12 hours after administration of the alkaloid showed a definite centrilobular necrosis and haemorrhage, so that in places there appeared to be pools of blood. The liver cells, though necrotic, were still *in situ*, and it was thus felt that the appearance was in fact a haemorrhage, and not a dilatation of sinusoids following on the removal of dead liver cells.

At 15 hours infiltration of the necrotic zone by polymorphs was first seen. This feature was noted in 9 out of 20 rats killed between 15 and 36 hours. At 21 hours disappearance of necrotic liver cells was obvious.

At 36 hours a vascular lesion similar to that described by Davidson (1935) in rats was first observed. It occurred in 10 out of 15 rats killed between 36 and 72 hours. The central and hepatic veins were often lined by a layer of large mononuclear cells, which Davidson considered to be swollen endothelial lining. In our animals these cells showed evidence of phagocytosis, and we therefore considered that they could perhaps be macrophages. This lesion was seen also in other experiments, occurring from 1½ to 12 days after administration of retrorsine. A further experiment with trypan blue was performed to elucidate this point and is described later.

From 40 to 72 hours after administration of the drug all the necrotic tissue in the central zones was absorbed, leaving sharply defined cyst-like spaces around the central veins (Fig. 2). These are referred to in Table I as " cysts."

Experiment 3 : Four rats were given 1 ml. of 1 per cent trypan blue subcutaneously on the first and the fourth days of the experiment, and retrorsine hydrochloride 1 mg. per 10 g. body weight orally on the fourth day. One rat died on the seventh day of the experiment, and the remainder were killed on the tenth day.

All but one of the rats showed the typical haemorrhagic zonal necrosis seen in the previous experiments, and, in addition, a marked lesion of the central and hepatic veins (Fig. 3–5). This consisted of one, two and sometimes even more layers of mononuclear cells, many of which contained trypan blue and showed other evidence of phagocytosis. Some of the cells were flattened against the wall, suggesting a mere swelling of the endothelial lining; others were attached by a broad base, and still others by an elongated cytoplasmic stalk (Fig. 5). Many of these cells were lying free in the lumen and similar cells were seen in the surrounding tissue.

The rat which did not show zonal necrosis had no evidence of a vascular lesion.

Experiment 4 : Several experiments were performed in an attempt, by varying the dosage and the route of administration of the alkaloid, to produce a vascular lesion approximating to that seen in the human cases.

Thus, 5 rats were given by mouth 0·05 mg. retrorsine hydrochloride per 10 g. body weight daily for 31 days. Five further rats were given 0·1 mg. daily for 21 days. Another group of 5 rats was given 0·5 mg. orally on alternate days for 7 days, by which time all 5 rats had died. In only one of these experiments was a lesion demonstrated in the hepatic veins.

Nine rats were then given retrorsine hydrochloride 0·5 mg. per 10 g. body

weight orally on the 1st, 2nd, 3rd, 10th, 11th, 12th, 30th and 31st days of the experiment and killed on the 35th day. Of these rats 5 showed early fibrosis commencing around the central veins, but in none was a vascular lesion demonstrable. It was felt that a more prolonged and perhaps more frequent administration of the drug in this dosage might have produced a more advanced picture of hepatic fibrosis.

Experiment 5: Ten rats were each given one subcutaneous injection of retrorsine hydrochloride 1 mg. per 10 g. body weight. One rat was killed 6 days later and showed a zonal haemorrhagic necrosis. The remainder were killed after 21 days. Two showed ascites, and in one of these there was also a pleural effusion. In those with ascites there was regeneration of liver tissue and early fibrosis, but no lesion was found in the central or hepatic veins.

Experiment 6: *Senecio ilicifolius* was gathered from the Knysna district, prepared in various ways and fed to rats. The experimental work was limited by the amount of plant available. Eight rats were given a normal diet to which the dried plant had been added, so as to form 10 per cent of the daily ration; 10 rats were given daily an aqueous extract of the dried whole plant in varying dosages; 6 rats were given daily an alcoholic extract prepared from the dried seed-heads of the plant. In these experiments the animals lived from 21 to 84 days. The results of these procedures were very variable and may be summarized as follows: Only 4 rats showed centrilobular haemorrhagic necrosis of the liver. In one of these and in three rats in which there was no necrosis there was a mild degree of apparent endothelial proliferation in the hepatic veins such as that which has already been described. In 5 rats a similar lesion was present in the pulmonary veins, and haemorrhages into the lungs were much more marked than in the previous experiments. These pulmonary haemorrhages were present in all but 3 animals. Ascites was present in 9 rats, 3 of which showed a zonal liver necrosis.

DISCUSSION.

Watt and Breyer-Brandwijk (1932), in discussing the pathogenesis of senecio poisoning, concluded that it was not possible to state whether the primary lesion was in the vascular system or in the liver parenchyma. More recent work has

EXPLANATION OF PLATE.

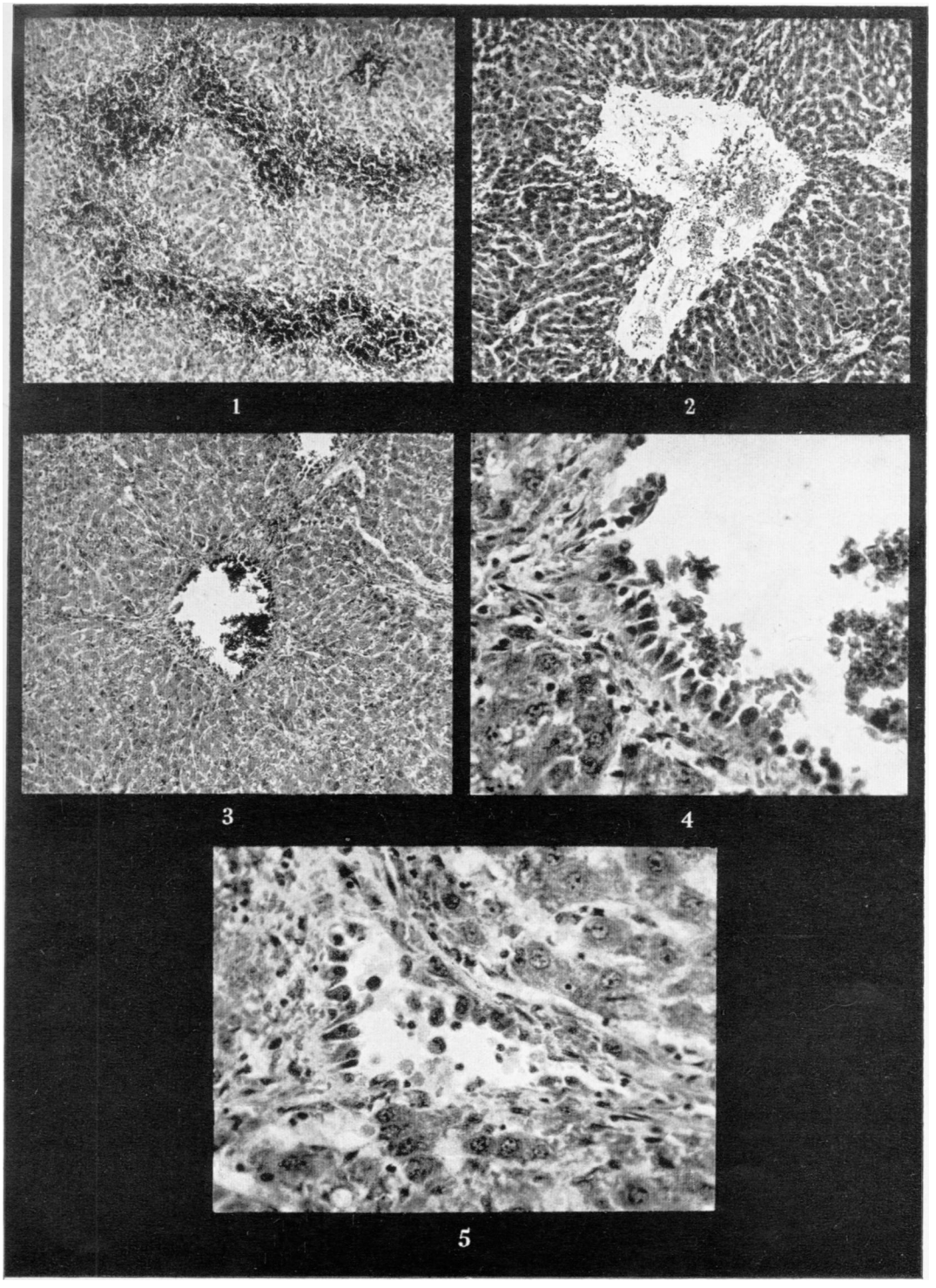
FIG. 1.—Liver of rat in Experiment 1 dying 2 days after administration of retrorsine. There is a zone of necrosis and haemorrhage around the central veins. Haematoxylin and eosin. × 100.

FIG. 2.—Liver from rat 40 in Experiment 2. The cells around the central veins have disappeared, leaving sharply defined cyst-like spaces. Haematoxylin and eosin. × 100.

FIG. 3.—Liver from rat in Experiment 3, killed six days after administration of retrorsine. A small hepatic vein is lined by a layer of mononuclear cells. Haematoxylin and eosin. × 100.

FIG. 4.—High-power view of the hepatic vein shown in Fig. 3. While some cells are lying free, others appear to be attached to the vessel wall by a cytoplasmic stalk. Haematoxylin and eosin. × 440.

FIG. 5.—Sublobular vein from the same animal as Fig. 2. The apparent attachment of the cells to the vessel wall is well seen, particularly to the left. Haematoxylin and eosin. × 440.



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not changed the position appreciably. Davidson (1935), for instance, while describing both vascular lesions and hepatic necrosis, considers the former to be the primary. Rosenfeld and Beath (1945) come to the same conclusion. Harris, Anderson and Chen (1942), on the other hand, stress the liver necrosis, although they describe sinusoidal congestion and haemorrhage into cell cords.

The almost simultaneous appearance of parenchymal necrosis and haemorrhage in our second experiment suggests that, contrary to Davidson's view, there is a primary toxic effect both on the liver cells and on the vascular system. Indirect evidence in favour of this latter is the presence of haemorrhages in situations other than the liver observed by us and by other workers (Cushny, 1910 ; Rosenfeld and Beath, 1945). The early haemorrhages in the liver appear to result from rupture of the central and hepatic veins, but such disruption of the vessel walls as is seen could, it is felt, have been produced artificially in the preparation of the sections. The only constant and convincing vascular lesion is the apparent endothelial proliferation described in our second experiment. The lesion corresponds very closely to that noted by Davidson. He considered it to be a proliferation of the endothelial lining of central and hepatic veins. Alternatively, the cells lining the affected vessels might, especially in view of their phagocytic power as shown in Experiment 3, be macrophages swept in from the surrounding tissues. Their appearance, however, strongly suggests that they are produced locally by the vascular endothelium, and according to Gillman (1950, personal communication) the endothelium of the central and hepatic veins can be phagocytic. Whatever the origin of these cells, they would appear to be capable of blocking the vessels, and it is possible that, although we have not demonstrated such a further development of the lesion in our experiments, they may eventually be replaced by fibrous tissue. In this way the appearance seen in the human cases, and probably in horses (Theiler, 1919 ; de Kock, du Toit and Steyn, 1931), could be produced. At this late stage any primary damage to the liver parenchyma would be masked by the secondary effects of the vascular lesions. Whether such primary parenchymal damage occurs in man and horses it is not possible to say, for the earliest stages of the process in these species have not been described.

Finally, we have shown that a protein-deficient diet markedly enhances the effect of retrorsine. This may have a bearing on the aetiology of the human disease, as all the cases described in our previous paper came from families in poor circumstances.

SUMMARY.

1. The hepatic lesions produced in rats by retrorsine, a senecio alkaloid, are described.

2. The primary effect appears to be both on the liver parenchyma and on the central and hepatic veins, so that the picture of a centrilobular haemorrhagic necrosis is produced.

3. At a slightly later stage an apparent proliferation of the endothelium of the central and hepatic veins is seen.

4. The possible relation of the experimental to the human lesions is discussed.

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