Development of Hepatic Tumors in Rats following Ingestion of Senecio longilobus

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SUMMARY

Prolonged administration of dried Senecio longilobus in the diet of rats led to necrosis and regeneration of liver cells with hypertrophy of some cells and nuclei, bile duct proliferation, and neoplasia of parenchymal cells. Additional changes included pulmonary arteritis and pulmonary adenomatosis. The highest tumor yield was obtained by alternate weekly feeding for 1 year of a diet containing 0.5% Senecio and a Senecio-free diet. Liver tumors developed in 22 male and 6 female rats. In 14 rats, sections showed invasion of contiguous veins. In 7 rats there were 2 distinct primary tumors. In 6 rats, there were intrahepatic metastases; 3 of these also showed pulmonary metastases, and several branches of the pulmonary arteries of another were plugged by proliferating tumor cells. Two rats had hepatic angiosarcomas with multiple nodules.

INTRODUCTION

Hepatic cirrhosis in livestock had long been known to follow ingestion of some species of Senecio when, in 1911, Cushny (4) reported production of hepatic necrosis in cats and rats with alkaloids from Senecio latifolia. Studies by several chemists of Senecio (pyrrolizidine) alkaloids during the late 1930's and early 1940's enabled Chen to obtain samples of 15 recently isolated alkaloids for pharmacological study. Single doses of 14 alkaloids produced hepatic necrosis in mice, and repeated doses caused hypertrophy of liver cells and nuclei (7, 8), a change now recognized as characteristic of Senecio poisoning. We stopped experimentation because of the high early mortality rate and a maximal survival of only 10 weeks, but we were stimulated to resume by the report of Cook et al. (3) of liver tumor induction in 3 rats by alkaloids from Senecio jacobea.

Since our supply of alkaloids was small, we obtained batches of dried Senecio longilobus in 1952 and 1953. With this material, we produced hepatic cirrhosis in sheep (9) and began studies with rats.

MATERIALS AND METHODS

The stems and leaves of the plants were ground and incorporated into our stock rat diet at levels ranging from 0.25 to 5%. Harlan rats of both sexes weighing 100 to 120 g were used. During the first 6 years of our studies, the rats were caged in groups of 4 until deaths diminished the number per group. Cage bottoms were of heavy wire woven in a coarse mesh that permitted free passage of feces. The animal quarters were air conditioned. Water was supplied from bottles with copper drinking tubes; the copper was later replaced by stainless steel. The food supply was replenished as needed. In some experiments, the plant-containing food was offered on alternate weeks, and plant-free food was given during the intervening weeks. In other experiments, exposure to the plant-containing food was continuous. Since some rats wasted much food, we prepared a pelleted diet. This entailed addition of a binder and water and drying after the formation of pellets.

Most rats were allowed to die spontaneously. Some were eaten by cagemates, and some were badly autolyzed when found. Tissues of 295 rats were examined microscopically. Discovery of microscopic liver tumors in 5 rats was reported in 1956 (6).

In 1962, we began a 2nd series of tests with the plants collected in 1953, modifying treatment in light of the results of the preceding experiment. The 1st group of 50 males and 50 females received continuously a diet containing 0.75% S. longilobus. Deaths began to occur rapidly after 1 month, and the longest survival time was 131 days. The concentration was reduced to 0.5% for the 2nd group of 50 males and 50 females. Survival times were better, but only 4 rats lived more than 200 days. The 3rd group (R 3-64) of 40 males and 40 females received a 0.5% Senecio diet for 1 month and then a Senecio-free diet for 2 weeks. This cycle was repeated for 1 year; thereafter the rats were given our colony diet. There were 10 male and 10 female controls; the number was small, but there was available for comparison a large number of rats from other long-term studies. The 4th group (R 7-65) of 50 males and 50 females was given a 0.5% Senecio diet for 1 week and then a Senecio-free diet for 1 week; the diets were alternated thus 27 times before Senecio feeding was stopped. An improved food container permitted the use of nonpelleted food. Water was supplied through pipes fitted with brass nipples and attached to a reservoir. The rats were housed individually in cages of the same general type as were used before. Weight of the females at the outset averaged 75 g, and that of the males averaged 83 g. Tissues of 320 of the Senecio-fed rats were examined microscopically.

RESULTS

Pulmonary edema was often present in rats that died during the first 3 months, but it diminished in frequency and severity.
thereafter; it was often associated with hydrothorax and, somewhat less often, with ascites. In some instances, edema appeared to be the immediate cause of death. Occasionally during the 2nd month, but more often later, foci of organizing pneumonia were seen. These eventuated in patchy fibrosis. Pulmonary congestion and some intraalveolar hemorrhage were often associated with pulmonary edema. In a few rats, hemoglobin crystal formation in alveoli followed hemorrhage. Focal extension of cuboidal or columnar epithelium out to line groups of alveoli was seen in many rats (Fig. 1) in association with organizing pneumonia and pulmonary arteritis. This epithelial proliferation (adenomatosis) was first seen after 2 months, but it was rare during the first 6 months. In the 2nd series of experiments, the overall incidence was 7%, and the incidence after 300 days was 31%. With increasing age, the number and size of these foci per lung increased, and in a few instances they involved a major portion of the lung tissue. Often the alveoli lined with columnar cells contained mucus, and sometimes small numbers of leukocytes were present. In 3 rats, metaplasia of these cells to the squamous type occurred.

Pulmonary arteritis was common in rats that died early. In the 2nd series of experiments, the incidence was 50% in rats dying before 100 days, 20% in those dying between 100 and 300 days, and 6% in those dying after 300 days. Arteritis involved vessels of all sizes, from small arterioles to the largest arteries (Figs. 2 and 3), but not all were involved uniformly or to the same degree. In arterioles, there was commonly hyaline necrosis with appreciable thickening of the media; sometimes there was slight leukocytic infiltration of the adventitia. In larger arteries, hyaline necrosis of the media was less common, but medial thickening with cell proliferation was common. Infiltration of all 3 layers by macrophages, polymorphonuclear neutrophils, and lymphocytes was common. Intimal thickening with cellular proliferation was pronounced in some of the larger arteries, resulting in much diminution of the lumen. In a few instances, large arteries contained thrombi (Fig. 3); thrombosis of small vessels was fairly common. Often leukocytes were absent from the walls of diseased arteries of rats that had been on test the longest.

In some rats, s.c. edema and ascites became conspicuous. The albumin and globulin contents of the blood were determined in 7 such rats and in 2 without ascites but with damaged livers. The albumin:globulin ratio was reversed in all 9. Expansion of the series was deemed unnecessary.

Occasionally, hemorrhage occurred into the s.c. and retroperitoneal tissues. A few rats bled into the vertebral canal and became paraplegic.

Liver cell necrosis was seen in nearly one-fifth of the rats that died during the 1st 3 months but was much less common thereafter. Necrotic cells were usually few and were often obscured by sinusoidal congestion. Occasionally, necrosis involving all or nearly all of 1 or more lobules was seen; sometimes this was associated with thrombosis of a vein, and in a few animals infarction of an entire lobe had occurred. After several months, many livers were reduced in size, although necrotic cells were not seen; however, some or practically all parenchymal cells and nuclei were hypertrophic (Fig. 4); in some instances, nearly all cells of some lobules had vanished. Cellular hypertrophy began after about 2 months and increased in incidence and degree thereafter. Regeneration of liver cells sometimes led to formation of nodules surpassing 1 cm in diameter. Proliferation of small and medium-sized bile ducts (Figs. 4 and 5) began during the 3rd month and increased in frequency, but the incidence never surpassed 60% of the rats. In some livers, bile duct proliferation was intense; in a few, small cholangiocystomas formed. Fibrosis occurred in some livers, but was never heavy; regenerative nodules were sometimes surrounded by a thin collagenous band.

After a short exposure to Senecio, some livers were dark reddish brown and friable but of normal size. Later, they became smaller; some were of normal color, some were light brown, and some were greenish brown. Diminution in size did not always affect all lobes equally. Some had become granular or even nodular because of regeneration of liver cells; this was rarely evident before 3 months. In the 1st series, none of the nodules was grossly, clearly neoplastic. Sections were made so as to include as many nodules as possible, and several sections were made of each block when a neoplasm was suspected. Most of the nodules were clearly regenerative, but there were many that suggested neoplasia (Fig. 5). Some actually may have been early tumors, but in the absence of incontestable evidence it seems best to classify them as nonneoplastic.

In 4 male and 2 female rats of the 1st series that had been on treatment for 133 to 446 days, hepatomas 1 to 3 x 5 mm in diameter were found adjacent to veins; the cells were well differentiated and arranged in cords 1 to 3 cells wide and, in 1 case, 1 to 6 cells wide. In all cases, identical cells were growing within the vein attached to its wall. An example is depicted in Fig. 6. In 5 rats, the invasive cells were traced in 3 to 6 sections, and, in 1, 2 sections of a 2nd distant vein contained adhering tumor cells next to a neoplastic nodule. Only 1 liver section was available in the 6th rat; it contained 2 small tumors with venous invasion. An additional male rat that had been on test for 479 days had a rounded 2-mm tumor composed of well-differentiated liver cells of uniform size in cords 2 or 3 cells wide; there was no venous invasion. The livers of all 7 rats contained multiple regenerative nodules.

In the 2nd series, the tumor incidence was higher, and the majority of tumors were easily distinguished from regenerative nodules on gross examination. In Group R 3-64, 23 rats lived more than 200 days, and 3 males and 1 female had hepatocarcinomas after 428 to 657 days. The 1st rat had a median lobe tumor (Figs. 7 and 8) 4.5 x 4.5 x 3 cm in size, with much central necrosis and multiple gray nodules up to 4 mm in diameter in the right and left lobes. With 1 exception, these resembled the large tumor histologically, and in several sections portal veins were plugged with proliferating tumor cells (Fig. 9), as were branches of the pulmonary arteries. The 2nd rat had a 2-cm tumor in the median lobe (Fig. 10), multiple nodules in the other lobes, and metastatic nodules ranging up to 8 mm in diameter in the lungs (Fig. 11) associated with bloody pleural fluid. One of the smaller liver nodules was a 2nd primary, while the others resembled the larger tumor. The 3rd rat had two 5-mm hepatocarcinomas of different histological appearance, as well as mesotheliomatous nodules all over the peritoneal surfaces. The 4th rat had a single 6-mm liver tumor. Another rat had peritoneal mesotheliomatosis without hepatoma.
The regimen in Group R 7-65 was the best; 47 rats lived more than 200 days, and 14 males and 3 females had malignant liver tumors after 217 to 470 days; 16 of these had hepatocarcinomas, and 1 had 3 angiosarcomas (example in Fig. 12). There were no cholangiocarcinomas. The tumors ranged from 0.3 to 1.3 cm in diameter. Eight rats had hemoperitoneum. Two rats had pulmonary metastases, and 4 had hepatic metastases. Four rats had 2 primary liver tumors. In 8 rats, the sections showed venous invasion from contiguous tumors. Of the 9 rats in which venous invasion was not seen, 1 had pulmonary metastases; 1 had 3 small masses of tumor cells in the blood in the right ventricle that could not have been artifactual; 1 had hepatic metastases; 2 had cords up to 10 cells wide, and 3 others had cords up to 5 cells wide (one of the latter also had multiple angiosarcoma); the remaining rat had the previously mentioned angiosarcomas.

The survival time by 100-day periods of all rats that were examined histologically, and the sex and exact survival times of liver tumor-bearing rats are given in Table 1.

**DISCUSSION**

In differentiating between hepatic regeneration and neoplasia, we have found the criteria of Long et al. (16) helpful. The possibility that some of the liver tumors were spontaneous can be dismissed readily; spontaneous liver tumors in Harlan rats are very rare, and we have seen only 1. Moreover, the tumors arose in livers that showed the characteristic effects of damage by pyrrolizidine alkaloids. The greater predilection of male rats to liver tumor development is striking and has been observed by Schoental (18) also.

A few rats developed extrahepatic tumors of types that we see occasionally arising spontaneously; there is no reason to consider them Senecio-induced. In our experience, peritoneal mesotheliomas are rare; we have seen them in 2 other rats not given Senecio. In the absence of a plausible Senecio-related mechanism for their genesis, we must consider them coincidental.

Others have seen development of pulmonary adenomatosis (5, 13, 14, 23) and pulmonary arteritis (1, 10—15, 17, 23) following administration of pyrrolizidine alkaloids. Five rats with liver tumors had pulmonary adenomatosis, but none had pulmonary arteritis. This suggests that pulmonary arteritis does not permit longevity and that adenomatosis has little effect thereon or continues to develop as rats age; data given earlier on the incidence of these lesions support these inferences.

Cook et al. (3), and Schoental et al. (19—21) have reported development of liver tumors in a total of 34 rats following administration of pyrrolizidine alkaloids; 2 rats had pulmonary metastases. The only confirmatory publication to date is that of Campbell (2), who reported finding malignant liver tumors in 3 chickens fed a mixture of seneciphylline and jacobine for intervals of 67, 79, and 147 days.

In 2 experiments, Sedlmeier et al. (22) failed to produce liver tumors in rats by giving Senecio vulgaris; in 1, the exposure was for 3 months, and in the other it was for various intervals up to 6 months. They reject the concept that Senecio alkaloids have a direct carcinogenic action. We cannot agree that these experiments disprove the oncogenicity of Senecio alkaloids; they merely fail to demonstrate it. A longer study might well have given positive results.
Although we used a *Senecio* plant rather than a pyrrolizidine alkaloid, we think it reasonable to consider our results as confirmatory of those of Schoental et al.

REFERENCES

Senecio-induced Hepatomas

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