

Islet Cell Tumors of the Pancreas Found in Rats Given Pyrrolizidine Alkaloids from *Amsinckia intermedia* Fisch and Mey and from *Heliotropium supinum* L.

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SUMMARY

One adenoma and one adenocarcinoma of the islet cells and one adenoma of the exocrine pancreas were found in 3 out of 15 rats given a single dose (500 to 1500 mg/kg body weight) of pyrrolizidine alkaloids from *Amsinckia intermedia* Fisch and Mey (Boraginaceae), tarweed, a plant known to cause livestock losses from liver damage in the United States.

Among rats treated with *Heliotropium supinum* L. (Boraginaceae), a hepatotoxic Ethiopian plant (known to be used in East Africa by women after childbirth), one islet cell adenoma was found in one out of six rats that were given a single dose of its crude alkaloidal fraction (300 mg/kg body weight) and one adenocarcinoma in one out of two rats that had the dried plant *H. supinum* in their diet for 1 month.

The five rats with pancreatic tumors were 26 to 31.5 months old when killed. Such tumors have not been noticed among control rats of similar ages.

INTRODUCTION

Pyrrolizidine (*Senecio*) alkaloids have been known to be hepatotoxic for more than half a century (6) and certain among them have been found to induce primary liver tumors in rats (14). Lung lesions and cor pulmonale follow more frequently the alkaloids from *Crotalaria* plants. Although enlarged spleens and edema of the pancreas have been seen to accompany severe liver lesions after PA² (13, 14), tumors of the pancreas have not been reported till now.

In the course of an investigation of the effects of the PA from *Amsinckia intermedia* Fisch and Mey (Boraginaceae, fiddle-neck, tarweed), a plant reported to cause poisoning and liver damage in livestock in the United States (11, 18), large doses of its alkaloids (5) (Chart 1) of the order of 1 g/kg body weight and higher had to be used in order to induce liver and lung lesions similar to those described after other PA (Refs. 8, 16; unpublished results).

Similarly, relatively high dosage is required to reveal the

hepatotoxic action of alkaloids from *Heliotropium supinum* L. (Boraginaceae) (3). Plants of this species growing in Australia have been shown to contain at least 5 open ester alkaloids (4) (Chart 2).

MATERIALS AND METHODS

Weanling male white rats randomly bred in the M.R.C. Laboratories, Carshalton, England, from the Porton-Wistar strain were used and were given the normal diet of MRC 41B (2) and water *ad libitum*. The rats were weighed at the beginning of the experiments and at monthly intervals or more often. All the animals that died or that were killed by coal gas when they appeared ill were autopsied; the liver, lungs, kidneys, stomach, and any other organs which seemed abnormal were fixed in Helly solution or in neutral 10% formol 0.9% NaCl solution; sections cut at 5 to 6 μ were stained routinely with hematoxylin and eosin; other stains were used when required.

A mixture of the alkaloids, intermedine and lycopsamine, from seeds of *A. intermedia* was given to weanling rats as a neutralized aqueous solution by stomach tube. Dried, ground plant (leaves and stems) of *H. supinum* L. from Ethiopia (15) was mixed with powdered diet (MRC 41B) (2) and fed to rats; another part of this plant material was extracted with ethanol, and the crude alkaloidal fraction was neutralized and given to rats by stomach tube.

RESULTS

Pancreatic islet tumors have not been noticed among our control rats or in other experimental groups in which the animals were allowed to live for as long as possible, often attaining the age of 2 to 3 years.

The pancreatic tumors found in the present experiments formed small nodules among the acini of the pancreas; some were not particularly conspicuous, and such nodules might escape detection unless especially sought. The details of the treatments and survival times of the rats in which the pancreatic tumors have been found are included in the chart legends.

The first rat in which an islet cell tumor of the pancreas was noticed belonged to a group of 15 male rats which were given, at the time of weaning, single doses of *A. intermedia* alkaloids (500 to 1500 mg/kg body weight) and which

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²The abbreviation used is: PA, pyrrolizidine alkaloids.

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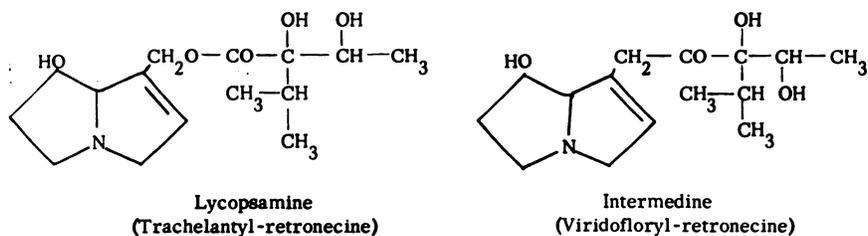


Chart 1. Structures of the alkaloids from *A. intermedia* Fisch and Mey.

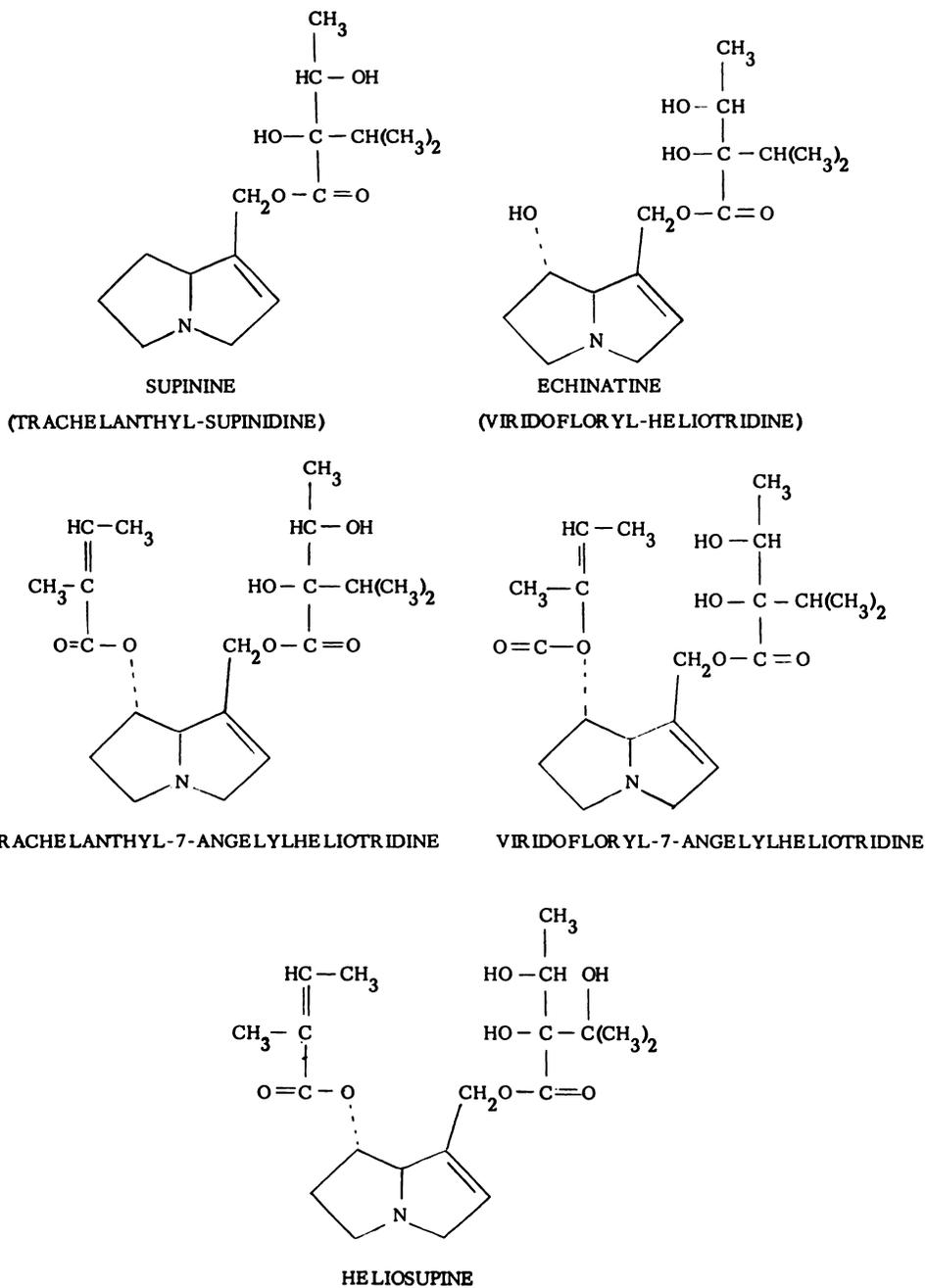


Chart 2. Structures of the alkaloids from *H. supinum* L.

survived without further treatment for more than 1 year after the dose. This rat was very large (more than 800 g body weight); it ate voraciously. At autopsy, much fat was present in the abdominal cavity, the testes were very small and atrophic, the liver showed only some variation in the size of the parenchymal cells, and a nodule (0.5 x 0.3 cm) was present in the pancreas; it proved to be an islet cell tumor (Fig. 1). The bladder was distended with papillary tumors, and a hemorrhagic adenoma was present in the pituitary. It is not unlikely that this islet cell tumor was functional.

Two other rats in this series were found to have pancreatic tumors, although these did not affect their body size. One had an islet cell adenocarcinoma with many enlarged cells showing bizarre nuclei (Fig. 2). Another rat had an adenoma of the exocrine pancreas.

Among rats treated with *H. supinum*, 2 islet cell tumors were found. One was a large, encapsulated adenoma (Fig. 3) found in 1 out of 6 rats that had been given, as weanlings, single doses of a crude alkaloidal fraction from *H. supinum* L. from Ethiopia (200 to 300 mg/kg body weight); all of these rats survived longer than 1 year after the dose. The other (Fig. 4) was found in 1 out of 2 rats that were given the dried, ground plant, 10% in powdered diet (MRC 41B), for 1 month after weaning, then kept on normal pelleted diet (MRC 41B) throughout their life.

DISCUSSION

Plants containing PA have been and are being used in various countries for medicinal purposes (7, 10, 17). *H. supinum* is known to be used in Tanzania by women after childbirth (15). In Germany, certain *Senecio* plants are being used for the treatment of diabetes (I. Eichler, personal communication). These plants can be tolerated in large doses. The hazard connected with the use of such herbs is still to be evaluated.

Tumors of the pancreas, particularly of the islet cells, are very rare in laboratory animals (12). The finding of 4 islet cell tumors among the small number of rats in our experimental series appears, therefore, significant.

Insulomas are the most frequent tumors encountered in cattle in Italy (1). *Senecio jacobaea* L. plants grow there abundantly as weeds in the pastures (G. Asdrubali, personal communication).

Our findings may be relevant for the etiology of pancreatic islet cell tumors in man. Until now, it has usually been surmised that cases of familial occurrence of islet cell tumors are of genetic origin (9). They could, however, be due to some such "natural products" as the PA. Members of a family usually partake of the same food and follow tradition with regard to herbal medicines.

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Fig. 1. Islet cell adenoma of the pancreas found in a male rat killed 25 months after a single intragastric dose of the alkaloids from *A. intermedia* Fisch and Mey seeds, 600 mg/kg body weight. H & E, X 161.

Fig. 2. Adenocarcinoma of the islet cells of the pancreas found in a male rat killed 27 months after a single i.p. dose of the alkaloids from *A. intermedia* Fisch and Mey seeds, 1500 mg/kg body weight, showing absence of encapsulation and spread of the tumor among the acini of the pancreas. H & E, X 31. *Inset*, variations in the size of the cells of the islet tumor. H & E, X 310.

Fig. 3. Encapsulated adenoma of the islet cells of the pancreas from a male rat killed 30.5 months after a single intragastric dose of the alkaloids from *H. supinum* L., 300 mg/kg body weight. H & E, X 15. *Inset*, cell type of the tumor. H & E, X 843.

Fig. 4. Adenocarcinoma of the islet cells of the pancreas from a male rat killed 28 months after the beginning of feeding of ground plant, *H. supinum* L., 10% in powdered diet MRC 41B, for 1 month, followed by normal diet (MRC 41B) for 29 months. Note the hemorrhage and disorganization of the structure of this tumor. H & E, X 19. *Inset*, the cell type of the tumor and a mitotic figure. H & E, X 1240.

