

period of high incidence of stem-end decay in citrus fruits, costs of treatments by the "stamp pad" method are almost negligible in comparison with losses that are frequently encountered in shipments of non-treated or improperly treated fruit. Shipments of fruits showing excessive decay cause indirect damage to grower and shipper alike in that consumer ill-will is often difficult to overcome.

This preliminary report is made with the thought that it may stimulate practical research leading to better control of plant diseases from the use of these chemicals and others like them. At the concentrations used, the manufacturers state that there is no danger from toxic residues.

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#### THE EXPERIMENTAL PRODUCTION OF HEPATIC CIRRHOSIS BY THE SEED OF AMSINCKIA INTERMEDIA<sup>1</sup>

FEEDING trials have demonstrated the seed of *Amsinckia intermedia*, Fisch. and Mey., also variously known as fiddle-neck, yellow burweed, yellow tarweed and tarweed, to cause necrosis of the liver parenchyma of swine, horses and cattle. The hepatic cells are replaced by connective tissue producing an extremely hard, cirrhotic liver. Similar changes also occur in the spleen, although to a lesser degree. In swine, which are most susceptible, and in cattle, which appear to be more resistant, the condition is known as "hard liver disease." In horses, in which any extensive destruction of the hepatic parenchyma is followed by cerebral disturbances, the condition is known as "walking disease" because of the tendency of affected horses to wander aimlessly.

*Amsinckia intermedia* grows profusely in the grain fields in certain semi-arid regions in Washington, Oregon, Idaho and California. Because of its irritant character the plant is not grazed by live stock. The seeds are heavy and are harvested with the grain. They are non-irritant and possess a pleasant nut-like flavor and at first are readily eaten by live stock. After animals have been made acutely ill, they tend to avoid the seed.

All of nine pigs, all of three horses and one of three calves fed wheat screenings rich in the seed of *Amsinckia intermedia* developed hepatic cirrhosis with symptoms characteristic of those observed in animals on farms where the plant grows abundantly. An alcoholic extract, representing approximately one pound of the seed, killed a 30-pound pig in less than 12 hours. The liver parenchyma was badly damaged. Further feeding trials have shown the toxic principle to be

<sup>1</sup> Published as Scientific Paper No. 427, Agricultural Experiment Station, State College of Washington.

contained in the petroleum ether insoluble fraction of such extracts.

While *Amsinckia intermedia* has been known to produce mechanical injuries,<sup>2</sup> and other members of the Borage family have been described as urticarious,<sup>3</sup> no member of this family previously has been shown to be toxic.

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#### AN EFFECT OF BENZEDRINE SULFATE ON CHICKS

MANY of the effects of the synthetic drug benzedrine sulfate (benzyl methyl carbinamine sulfate) are similar to those of epinephrine. It is used in inhalers to shrink the nasal mucosa, and has been used in the treatment of narcolepsy. When day-old Rhode Island Red chicks are given 1 mg of benzedrine sulfate subcutaneously they show visible effects of the drug in seven to nine minutes; mouths open, legs become unsteady so that the body falls forward, and the wings droop and spread. The most remarkable reaction is the incessant twitter or "singing," roughly 220 notes per minute, which continues for fifteen minutes to half an hour. All the visible effects disappear in about forty-five minutes.

When larger doses (3.5 mgs) are used the effects appear in two or three minutes and the rate of twittering is increased up to 300-330 notes per minute. Apparently the drug is eliminated rapidly as the chicks return to normal inside of an hour.

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#### ARE PECTIC SUBSTANCES PRECURSORS TO LIGNIN?

RED clover (*Trifolium pratense*), a lignified tissue, may contain pectic substances and lignin in practically equal amounts. In general, lignified tissues contain very small percentages of pectic substances and considerable lignin, while in non-lignified tissues the opposite is true. For these reasons, it has been thought that lignin is formed from pectic substances in the plant. In red clover, however, the proportion of these two substances does not indicate that one is the precursor of the other; however, it does support Bailey's<sup>1</sup> contention that, because of the difference in cell structure, it is not essential to account for the various proportions of these substances in different plants on the basis of transformations. Table 1 illustrates the percentages of pectic substances and lignin

<sup>2</sup> L. H. Pammel, *Vet. Med.*, 21: 220, 1926.

<sup>3</sup> Walter Conrad Muenscher, "Poisonous Plants of the United States," New York: Macmillan Company, 1939.

<sup>1</sup> I. W. Bailey, *Ind. Eng. Chem., Ind. Ed.*, 30: 40-47, 1938.

TABLE 1

	Pectic substances	Lignin
	Per cent.	Per cent.
Red clover ( <i>Trifolium pratense</i> )....	5.3 -7.8	6.8- 9.5
Kentucky blue grass ( <i>Poa pratensis</i> )	0.7 -1.0	7.7-10.6
Timothy ( <i>Phleum pratense</i> )*.....		10.4
Italian rye grass ( <i>Lolium italicum</i> )†		10.4
Other grasses‡ .....	0.82-2.10	
<i>Leaves:</i>		
Beans§ .....	6.5 -8.9	.....
Cabbage§ .....	7.4	.....
Hyacinth§ .....	9.2	.....

\* Max Phillips, *Jour. Assn. Official Agr. Chemists*, 16: 476-479, 1933.

† A. G. Norman, *Jour. Am. Soc. Agron.*, 31: 751-760, 1939.

‡ Harold William Buston, *Biochem. Jour.*, 28: 1028-1037, 1934.

§ *Ibid.*, 29: 196-218, 1935.

in red clover and various grasses as hay and in certain leaves. The results are expressed on a dry matter basis.

In view of recent developments, perhaps the pectic substances when present to this extent in a feed have a value other than a calorific one.

Seasonal trends of the content of pectic substances, hemicelluloses and lignin in red clover and Kentucky blue grass, together with a discussion of their relationships, will be published later.

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## SPECIAL ARTICLES

### THE PRODUCTION OF TUMORS BY INJECTION OF A CARCINOGEN INTO THE AMNIOTIC FLUID OF MICE<sup>1</sup>

VERY few, if any, attempts have been made to induce tumors by subjecting embryonic tissues to the action of carcinogenic agents. This report is to record the production of neoplasms in mice exposed to 1:2:5:6 dibenzanthracene as embryos *in utero*.

The mice used were F<sub>1</sub> animals produced by crossings of Caracul ♀ × P stock ♂. The Caracul mice were inbred to the F<sub>4</sub> generation, while the P stock males were of the F<sub>13</sub> generation. These F<sub>1</sub> embryos were heterozygous for the coat characters Caracul, a dominant waved mutation, albinism, pink-eye, extreme dilution and Agouti. It was hoped that with such animals possible somatic mutations could be observed. At the stage used in these experiments (14-day stage), the epidermis is composed of a single row of ectodermal cells and the Malpighian layer or *stratum germinativum*, which later gives rise to the hair follicles, has not yet differentiated.<sup>2</sup>

The vaginal plug method was used to detect fertilization. Fourteen days after plug formation the female was anesthetized with nembutal, the uterus exposed and approximately 0.5 minim of 1:2:5:6 dibenzanthracene in olive oil injected through the uterine wall directly into amniotic fluid of each embryo present. The dosage of carcinogen used was 4 mg of 1:2:5:6 dibenzanthracene per cubic centimeter of purified olive oil. Injections were made with a 27-gauge hypodermic needle. Abortion, resorption or birth of dead young were common. Premature birth occurred generally 17 days after plug formation so that the carcinogen was in contact with embryonic tissue for only 3 days in the majority of cases. Complete necropsies were done on all animals and histo-

logical sections made of all abnormal growths. (See Table 1.)

TABLE 1

FATE OF MICE OF EXPERIMENTAL SERIES A INJECTED WITH 1:2:5:6 DIBENZANTHRACENE AS EMBRYOS *in utero*. ABSORPTION TIME REPRESENTS TIME FROM INJECTION OF CARCINOGEN INTO FLUID UNTIL PARTURITION. SEE TABLE 1 FOR TYPE REACTION IN THE LUNG.

Animal	Absorption time (days)	Age at time of necropsy (days)	Tumors
♀ F <sub>1</sub> 122	6	197	No tumor
♀ F <sub>1</sub> 121	4	169	Carcinoma lung +++
♀ F <sub>1</sub> 120	3	223	Carcinoma lung ++
♀ F <sub>1</sub> 119	3	196	Carcinoma lung +++
♀ F <sub>1</sub> 118	3	196	Carcinoma lung +++
♀ F <sub>1</sub> 117	3	196	Carcinoma lung ++
♀ F <sub>1</sub> 116	5	186	Carcinoma lung +++
♀ F <sub>1</sub> 115	5	186	Carcinoma lung +++
♀ F <sub>1</sub> 114	5	186	Carcinoma lung +++
♀ F <sub>1</sub> 113	5	186	Carcinoma lung +++
♀ F <sub>1</sub> 112	3	196	Carcinoma lung +++
♀ F <sub>1</sub> 111	3	196	Carcinoma lung ++
♀ F <sub>1</sub> 110	3	224	Carcinoma lung ++
♀ F <sub>1</sub> 109	3	224	Carcinoma lung ++
♀ F <sub>1</sub> 108	3	196	No tumor
♀ F <sub>1</sub> 107	3	196	No tumor
♀ F <sub>1</sub> 106	3	183	Carcinoma lung +++ ; fibrosarcoma
♀ F <sub>1</sub> 105	3	155	Carcinoma lung +++
♀ F <sub>1</sub> 104	3	224	Carcinoma lung +++ ; carcinoma liver
♂ F <sub>1</sub> 103	3	224	Carcinoma lung +++
♂ F <sub>1</sub> 102	3	223	No tumor
♂ F <sub>1</sub> 101	3	223	Carcinoma lung ++
♂ F <sub>1</sub> 100	3	223	Carcinoma lung +++

There were but 23 animals of this experimental group which lived to maturity. Nineteen or 82.6 per cent., of these animals showed primary carcinoma of the lung when necropsied at an average age of 200.3 days. Five of these mice showed an extreme neoplasia involving most, or all, of the lung. Unfortunately, none of the experimental series was killed earlier than 155 days, at which time ♀ F<sub>1</sub> 105 showed numerous large nodules in the lungs. Two animals showed multiple tumors. A tumorous growth above the skull appeared in ♀ F<sub>1</sub> 106 at 163 days of age. Histological sections showed this to be a fibrosarcoma. At necropsy, 183 days, the lungs of this animal also showed extreme neoplasia. ♂ F<sub>1</sub> 104 had extreme carcinoma of the

<sup>1</sup> The author is holder of a Finney-Howell Foundation Medical Research Fellowship.

<sup>2</sup> S. C. Reed and G. Sander, *Growth*, I: 194, 1937.