TABLE 1

	Pectic substances	Lignin
	Per cent.	Per cent.
Red clover (*Trifolium pratense*)	5.3 –7.8	6.8– 9.5
Kentucky blue grass (*Poa pratensis*)	0.7 –1.0	7.7–10.6
Timothy (*Phleum pratense*)*		10.4
Italian rye grass (*Lolium italicum*)†		10.4
Other grasses‡	0.82–2.10	
Leaves:		
Bean§	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[1]

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 uteri.*

The mice used were F_1 animals produced by crossings of Caracul ♀ × P stock ♂. The Caracul mice were inbred to the F_4 generation, while the P stock males were of the F_{13} generation. These F_1 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.[2]

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 histological 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 uteri.* 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_1 122	6	197	No tumor
♂ F_1 121	4	169	Carcinoma lung ++++
♀ F_1 120	3	223	Carcinoma lung ++
♂ F_1 119	3	196	Carcinoma lung +++
♀ F_1 118	3	196	Carcinoma lung +++
♀ F_1 117	3	196	Carcinoma lung ++
♂ F_1 116	5	186	Carcinoma lung +++
♂ F_1 115	5	186	Carcinoma lung ++++
♂ F_1 114	5	186	Carcinoma lung ++
♀ F_1 113	5	186	Carcinoma lung ++++
♂ F_1 112	3	196	Carcinoma lung +++
♂ F_1 111	3	196	Carcinoma lung +++
♀ F_1 110	3	224	Carcinoma lung ++
♀ F_1 109	3	224	Carcinoma lung ++
♀ F_1 108	3	196	No tumor
♀ F_1 107	3	196	No tumor
♀ F_1 106	3	183	Carcinoma lung +++; fibrosarcoma
♀ F_1 105	3	155	Carcinoma lung +++
♂ F_1 104	3	224	Carcinoma lung +++; carcinoma liver
♂ F_1 103	3	224	Carcinoma lung ++++
♂ F_1 102	3	223	No tumor
♂ F_1 101	3	223	Carcinoma lung ++
♂ F_1 100	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_1 105 showed numerous large nodules in the lungs. Two animals showed multiple tumors. A tumorous growth above the skull appeared in ♀ F_1 106 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_1 104 had extreme carcinoma of the

[1] The author is holder of a Finney-Howell Foundation Medical Research Fellowship.
[2] S. C. Reed and G. Sander, *Growth,* I: 194, 1937.

lung as well as carcinoma of the liver (see Table 1). It should be noted that in contrast to control animals all nodules in the lungs were easily visible with the naked eye.

In a second series (Series B), 0.5 minim of the same dosage dibenzanthracene in olive oil was injected intraperitoneally into F_1 mice 24 hours of age. Of this group necropsied at an average age of 180.8 days, 24 animals or 100 per cent. had carcinoma of the lung. Two animals also had fibrosarcomas in the region of the nape of the neck, although injections were made intraperitoneally.

There were two series of control animals. Twenty-nine mice received subcutaneous injections of the same dosage of dibenzanthracene when 2 months of age (Series C). Of this group, necropsied at an average age of 189.1 days, only 2 animals showed neoplasms, both carcinoma of the lung. In each case only a single nodule was detected under the dissecting microscope. Of 31 uninjected animals (Series D), necropsied at an average age of 228.4 days, only one animal had carcinoma of the lung, and this also was a single nodule detected by the dissecting microscope. All growths observed in the lungs were primary carcinomas. (See Table 2.)

TABLE 2

Series	Experiment	No. of mice	Average age at necropsy (days)	Number of tumors: Carcinoma lung	Number of tumors: Other tumors	Per cent. tumorous animals	Type reaction in lung
A	Injection of dibenzanthracene into amniotic fluid	11 ♀♀ 12 ♂♂	200.3	19	1 fibrosarcoma 1 carcinoma liver	82.6	6 ++ 8 +++ 5 ++++
B	Injection of dibenzanthracene into mice 24 hours of age (intraperitoneal)	11 ♀♀ 13 ♂♂	180.8	24	2 fibrosarcomas	100	4 ++ 6 +++ 14 ++++
C	Injection of dibenzanthracene into mice 2 months of age (subcutaneous)	16 ♀♀ 13 ♂♂	189.1	2	0	6.9	2 +
D	Uninjected controls	16 ♀♀ 15 ♂♂	228.4	1	0	3.2	1 +

+ = Single nodule. Detected in each case by use of dissecting microscope; ++ = few nodules, 5–10 visible with naked eye; +++ = numerous nodules, 10–20; ++++ = many nodules, 20 +, or tumor involving all, or most of, lung tissue.

It is of interest to note that the total maximum amount of dibenzanthracene present in the amniotic fluid, providing all remained subsequent to the injection, was but 0.125 mg per embryo. Since the average absorption time was only 3.5 days, it is quite probable that the minimum dosage required for tumor formation in the lungs of the F_1 mice under these conditions is considerably less than 0.125 mg. Previous reports[3,4] have indicated that as little as 0.1 mg of dibenzanthracene, in cholesterol-pellet form, would induce tumors at the site of injection in a very small percentage of animals.

No somatic mutations were observed among the 23 mice injected *in uteri* at the 14-day stage.

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ANAPHASE MOVEMENT IN ALLIUM CERNUUM

AT the first microspore division of many seed plants the orientation of the spindle at right angles to a wall makes it possible to determine the rate of anaphase movement for each group of daughter chromosomes. One spindle pole, at which the generative nucleus is later differentiated, appears to be pressed against a wall of the microspore; the other, at which the vegetative or tube nucleus is to be formed, extends into the cytoplasm. Thus at anaphase the generative group of chromosomes moves toward the wall, while the vegetative group moves away from the wall.

To determine the relative rate of movement of each group of chromosomes, measurements were made from permanent smears of *Allium cernnum* fixed in La Cour's 2BE and stained in crystal violet. Measurements were made from camera lucida drawings at a magnification of 3660×. Metaphases were measured from side view with respect to the distance from the wallward edge of the generative chromosomes at the centromere to the inner edge of the microspore wall. The thickness of the metaphase chromosome at the centromere was also determined. The sum of these two measurements is the distance from the centromeres of the vegetative chromosomes to the wall. Anaphases were measured from side view with respect to the distance between the two groups of centromeres and the distance from the generative centromeres to the wall. The distance from the centromeres of the vegetative chromosomes to the wall is the sum of these two mea-

[3] M. J. Shear and Egon Lorenz, *Am. Jour. Cancer*, 36: 201, 1939.
[4] F. W. Ilfeld, *Am. Jour. Cancer*, 26: 743, 1936.