

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.)

at the site of injection in a very small percentage of animals.

No somatic mutations were observed among the 23 mice injected *in utero* 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 cernuum* fixed in La Cour's 2BE and stained in crystal violet. Measurements were made from camera lucida drawings at a

TABLE 2

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

+ = 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

³ M. J. Shear and Egon Lorenz, *Am. Jour. Cancer*, 36: 201, 1939.

⁴ F. W. Ilfeld, *Am. Jour. Cancer*, 26: 743, 1936.

magnification of 3660 $\times$. 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-

surements. The anaphases measured were divided into four classes according to the distance between the centromeres. Class I includes anaphases in which the centromeres of the daughter groups of chromosomes are 2μ to 5μ apart; Class II 5μ to 8μ apart; Class III 8μ to 10μ apart; Class IV over 10μ apart. The average distance between the centromeres and the average distance from the generative centromeres to the wall was obtained for each class.

The distance traveled by each daughter group of chromosomes is the difference between its position at metaphase and its position at anaphase. At metaphase the generative centromeres are 3.9μ from the wall; the vegetative centromeres are 5.0μ from the wall (average of 50 measurements). In Class I the daughter groups of centromeres are 3.8μ apart; the generative centromeres are 3.4μ from the wall (average of 13 measurements). Thus when the daughter groups of chromosomes are 3.8μ apart the generative chromosomes have traveled 3.9μ less 3.4μ or $.5\mu$. The distance traveled by the vegetative chromosomes is 7.2μ less 5.0μ or 2.2μ . The ratio of distance traveled by the vegetative chromosomes to the distance traveled by the generative chromosomes is 4.4:1. The average distance traveled by each group of chromosomes at different anaphase stages and the ratio of vegetative chromosome to generative chromosome movement is shown for *Allium cernuum* in Table 1.

TABLE 1

	Meta- phase	Anaphase Class			
		I	II	III	IV
Average distance (μ):					
between centromeres ..	1.1	3.8	6.4	9.0	10.7
from gen. centromeres					
to wall	3.9	3.4	2.3	1.0	0.6
travelled by gen. chro-					
mosomes	..	0.5	1.6	2.9	3.3
travelled by veg. chro-					
mosomes	..	2.2	3.7	5.0	6.3
Number of measurements	50	13	33	40	53
Veg./Gen. ratio	..	4.4	2.3	1.8	1.9

It must be pointed out that the daughter groups do not merely travel different distances but travel at different rates; *i.e.*, it is not a case of the generative group being stopped by the wall while the vegetative group continues its movement. If this were so we would expect to find the distance traveled by each of the two groups to be the same until the generative group reaches the wall, then the daughter groups would be about 8.9μ apart (twice the distance from the generative centromeres to the wall plus the thickness of the metaphase chromosome). As shown in the table, this is not the case. When the daughter groups are 6.4μ apart the vegetative group has traveled 3.7μ ; the generative group only 1.6μ , and it is still 2.3μ from the wall.

Whether difference in rate of movement of the two groups of chromosomes results primarily from a

stretching of the anaphase spindle, as suggested by Geitler (1935),¹ or from a difference in time of development of the two spindle poles will be discussed in a later report which is concerned with anaphase movement in *Allium cepa*, *Panocratium illyricum*, *Tradescantia* and *Vicia faba*, as well as *Allium cernuum*.

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MULTIPLE GROWTH LAYERS IN THE ANNUAL INCREMENTS OF CERTAIN TREES AT LUBBOCK, TEXAS¹

A NOTABLE feature of the climate of Lubbock, on the High Plains of Texas, is the common occurrence of heavy frost after tree growth has begun for the season. These frosts are sufficiently intense to rupture the newly formed cells in the branches and leaders of many trees and to cause the formation of a partial or entire "frost ring" in the wood. Since the injuries must take place early in the spring, which is soon after growth begins, their presence marks the actual beginning of growth for the particular years. Definite frost injuries occurred in 1932, 1933, 1934, 1936 and 1938, slight and restricted injury in 1937, and no injury in 1935. These dates, established by examination of the wood, were verified by meteorological and vegetational records.

Once determined and dated, the frost injuries furnish a unique method of recognizing the true extent of wood formed during one year, because, with the injuries occurring as listed above, they register the beginning of each year's growth. This is commonly true for all years except 1935, in which temperatures did not sink sufficiently low to cause injury. Thus the method permits the determination of whether or not the trees studied form one or more "rings" per year.

All the trees reported upon here, except one, grew on the campus of Texas Technological College. Most of the work was done on the Arizona cypress and western yellow pine, but four other species were also examined. Many thin sections were made from different branches of the same tree, from different points along one branch axis, and, in two cases, from leaders.

Two anatomical observations are worthy of record at the present stage of the work: the number of growth layers in an annual increment, and the forms of the layers. In this connection the term "annual increment" refers to the total amount of wood formed during a complete growing season, and "growth layer" refers to a "ring" composed of early and late wood whose outer margin is sharply set off from the succeeding layer. An annual increment contains one or

¹ L. Geitler, *Planta.*, 24: 361-386, 1935.

¹ A paper read at the Alpine, Texas, meetings of the Southwestern Division of the American Association for the Advancement of Science, May, 1939.