

independent test of the postulated relation between the O antigens and the tumor-hemorrhage agents, we propose to examine this point in detail later.

We are unable at the present time to account for the absence of a demonstrable toxin in *Rhizobium*, *Phaemonas*, *Streptococcus varians* and *Rhodobacillus palustris*.

The two isolates of *Listeria* are the only gram-positive organisms observed to yield hemorrhage-toxins. The taxonomic position of this species is uncertain, yet *Erysipelothrix*, to which *Listeria* may be closely related,^{7,8} was inactive. The anomalous systematic position of *Listeria* has been recognized by Stanier and Van Niel.⁵

The lack of hemorrhage with *Cytophaga krzemieniewskæ* is not conclusive as only small

amounts of cell body were available. A study of more material and other members of the *Myxobacterales* is needed to determine whether the gram-negativity of this group is indicative of a relationship to gram-negative bacteria, if tumor-hemorrhage constitutes a criterion.

Conclusions. An extension was made of an earlier survey of bacterial substances inducing hemorrhage in implanted mouse tumors. It was found that with a few exceptions gram-negative forms induced tumor-hemorrhage, while this property, with one exception, was lacking in the gram-positives. An interesting example was the induction of hemorrhage by the photosynthetic gram-negative purple bacterium *Rhodospirillum rubrum*. It is concluded that toxins inducing tumor-hemorrhage, and probably bound up in the complex O antigens, are characteristic of gram-negative bacteria.

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Lung Tumors Following Intraperitoneal Injection of 1:2:5:6-Dibenzanthracene into Young Mice of Three Strains.

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Data have already been published¹ demonstrating inherited differences in degree of susceptibility to spontaneous and to tar-induced lung tumors in two inbred strains of mice when the criteria for susceptibility included not only the percentage of tumor-bearing animals per group but also the number of tumor nodules per individual. The same criteria revealed constitutional differences in susceptibility among 3 strains of mice observed after intraperitoneal injections of 1:2:5:6-dibenzanthracene into very young animals.² The present paper reports further data obtained after intraperitoneal injections, combined with the original observations made following this technic.

The three strains used, the Swiss, our branch of the Bagg albinos, and No. 1194,* differ in susceptibility to spontaneous neoplasms. The Swiss have the highest lung tumor incidence. In a sample of 448 mice (Table I), about one-half, or 46.1%, had tumor. This figure is based upon mice of all ages. However, tumor incidence increases with age, and, in groups of mice of this strain that have reached a year and a half or more, 80% have tumor. Tumors occur early in this strain, a growth having been observed in a mouse only 3 months of age. One of the characteristics of lung tumor is that the nodules may be multiple. In the Swiss strain, of those animals that have tumor, almost half have multiple nodules, the largest number of nodules observed in a single

¹ Lynch, C. J., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 186.

² Lynch, C. J., *Cancer Research*, 1941, **1**, 740.

* Source of material: Swiss-8, (8117, 8123); Bagg-L (1916, 1917); Strain No. 1194, (421, 453).

TABLE I.
Spontaneous Lung Tumor in 3 Strains of Mice.

Strain	No. of mice all ages	Lung tumor %	Earliest occurrence of tumor	Largest No. nodules per mouse
Swiss	448	46.1	3 mos.	13
Bagg	1382	26.3	5 mos.	9
1194	2660	4.6	11 mos.	7

individual being 13. The Bagg strain is less susceptible. Among 1382 animals of all ages, about a quarter, or 26.3%, had tumor. The growths do not appear quite so early—the youngest mouse to be found with tumor was 5 months old, and the largest number of nodules in any mouse was 9. The least susceptible of the 3 strains is No. 1194. Of 2660 mice of all ages, only 4.6% had lung tumors. Very few tumors are found in mice less than a year old. In this sample, only one mouse had tumor as early as 11 months. Even in old mice the incidence is low; it is only 17% in mice between 2½ and 3 years of age. Of the few animals with tumor, the large majority have a single nodule, and 7 nodules is the largest number found in one individual. In general, in the strains with the highest tumor incidence, tumors appear earlier and the number of nodules per individual is higher.

To induce lung tumors, a single intraperitoneal injection of 0.5 minim of a 0.4% solution of 1:2:5:6-dibenzanthracene in olive oil was given to mice 2 to 4 days old. The variation in age was due to an attempt to have the animals approximately the same size. This dose of 0.125 mg of dibenzanthracene is a large one for such small specimens and is probably the most effective for tumor induction. Counts were made of the nodules seen on the surface of the lung with a dissecting microscope.

The results of the injection are shown in Fig. 1. It is apparent that the method used is effective in inducing large numbers of tumor nodules. Mice from each of the strains, dying within a few days of the injection were not autopsied and are not recorded on the chart. A few representatives of each strain died before the 60th day and were autopsied, but none had tumor. Between the 65th and 75th day after injection, 12 mice of the Swiss strain died or were killed. All were found to have tumor, the number of nodules per mouse

ranging from 24 to 86. This is a much higher number than ever observed to arise spontaneously in these strains. No individual of the Swiss strain, coming to autopsy after this time was tumor-free. A number of mice belonging to the 3 strains died during the next 3 months and between the 180th and 210th day all remaining animals either died or were killed. In the Swiss strain the number of nodules per mouse had increased enormously, some animals having more than 150 tumor foci. In the upper ranges it is difficult to make accurate counts because the nodules coalesce, and the figures given here represent minimal values. Of 31 mice examined during the last 35 days of the experiment, only one individual had fewer than 84 foci, and the majority had many more. Of the Bagg strain, not as many animals were available for injection with the hydrocarbon. Autopsies of the few mice that died between the 110th and 160th day showed that all had lung nodules but not as many nodules per individual as found in the Swiss during the same period. Of the 12 Bagg mice that died or were killed during the last 35 days, all had tumor, and all but one had between 27 and 61 foci per mouse. Strain 1194 presented a very different picture. Of the 29 mice autopsied between the 175th and 210th day, only half (15) had tumor and no mouse during the entire experiment had more than 5 nodules.

It is evident from inspection of the last 35 days of the chart that in spite of a slight amount of overlapping, the mice of these 3 inbred strains fall into 3 distinct groups. The greatest spread is seen in the Swiss, less in the Bagg, and least in strain 1194. The first 2 strains mentioned both attained an incidence of 100% tumor mice and if the percentage of mice affected were the only criterion of susceptibility these 2 stocks would appear to be alike, but when susceptibility is judged by the num-

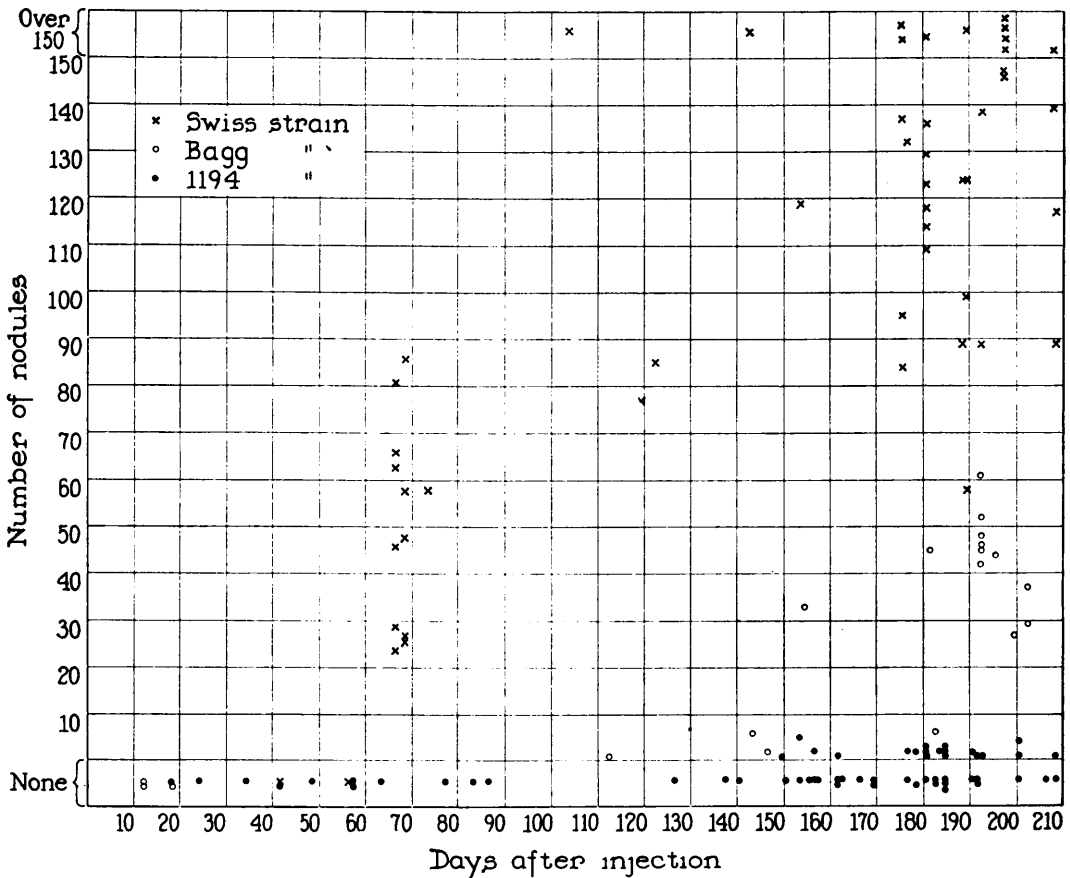


FIG. 1.

Showing the number of lung nodules per individual in mice of 3 strains injected intraperitoneally when 2-4 days old with 0.5 minim of a 0.4% solution of 1:2:5:6-dibenzanthracene in olive oil.

ber of nodules per individual strain differences are still apparent. The third strain, 1194, attained an incidence of 51.7%. Thus it is seen that the induced tumors appeared at a much earlier age than do the spontaneous growths, and they appeared in a much higher percentage at 6 to 7 months than ever observed in this strain at any age under ordinary laboratory conditions. However, the range of nodules per individual was no greater, in fact not quite as great, as it is in the spontaneous disease.

Since the differences noted are presented by three inbred strains, it is clear from the experimental results that differences in degree of susceptibility are inherited and that a single pair of genes could not account for the observed facts. At least 3 genes must be involved, and a larger number is not excluded.

That a number of genes are concerned in the inheritance of susceptibility to induced lung tumors has been found by Heston also. In the genetic analysis of the results of crossing certain of his strains of mice, it was shown that at least 4 and probably more pairs were indicated,³ and 2 susceptibility factors were located on 2 different chromosomes.⁴ Ander-vont's studies⁵ also have indicated more than one pair. The genetic relationships of our strains are being investigated further.

Summary. A single intraperitoneal injection of 0.5 minim of a 0.4% solution of 1:2:5:6-dibenzanthracene in olive oil was

³ Heston, W. E., *J. Nat. Cancer Inst.*, 1942, **3**, 69.

⁴ Heston, W. E., *J. Nat. Cancer Inst.*, 1941, **2**, 127.

⁵ Andervont, H. B., *J. Nat. Cancer Inst.*, 1940, **1**, 135.