Summary. 1. Trichina cysts did not calcify spontaneously *in vitro,* even when exposed to media with high $Ca^{++} \times P$ products. 2. When calcification was initiated *in vivo,* by administration of toxic doses of viosterol over a period of several days, incubation with serum *in vitro* led to a marked increase in the amount of calcification of the cysts. 3. Calcification of the cysts, when it occurred at all, occurred at $Ca^{++} \times P$ products well below the critical point for the calcification of rachitic cartilage. 4. The action of viosterol in predisposing to calcification, both *in vivo* and *in vitro,* is attributed to a toxic effect upon the cyst or the worm. 5. An enzyme which hydrolyzes glycerophosphate is present in trichina cysts. It is present in the absence of administration of viosterol, which did not affect either its concentration or its distribution. It is present in the cysts for several months before calcification usually occurs spontaneously *in vivo* in rats.

11742 P

Tissue Response to p-Amino Benzene Sulfonamide (Sulfanilamide) in Mice.

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With the greatly increasing use of the sulfonamide compounds in medicine in the past several years, a lively interest has arisen in regard to the effects of these drugs on the organism outside of their now well known activity in combating various types of infections. Numerous toxicological studies have therefore appeared on this subject. The somewhat equivocal relationship of p-amino benzene sulfonamide (sulfanilamide) to some of the aniline dyes suggested the possibility that the former might have mild carcinogenic properties similar to those reported for the aniline dyes,[1] and prompted the investigation described below. Subsequently, it came to the author's attention that a similar investigation had already been carried out by Lewis,[2] at the suggestion of Dr. E. K. Marshall. The results were subsequently confirmed by Zamecnik and Koletsky.[3] These

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1 Hueper, W. C., Wiley, F. H., and Wolfe, E. J., *J. Ind. Hygiene,* 1938, **20**, 46.

2 Lewis, M. R., *Am. J. Cancer,* 1938, **34**, 431.

3 Zamecnik, P. C., and Koletsky, S., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 391.

workers used pedigreed mice that were known to have a natural genetic resistance to neoplasms. Olive oil was the suspending medium used in both these investigations. Both reported that sulfanilamide was carcinogenically inert under these conditions. The following experiment was carried out under slightly differently controlled conditions and the preliminary results are herewith presented because they are at variance with those reported by the authors mentioned.

One hundred Swiss Albino mice were obtained from the Roscoe B. Jackson laboratory in Bar Harbor, Maine. These animals are not pedigreed but the natural incidence of cancer is low. An attempt was made to compare the action of sulfanilamide with 1:2:5:6 dibenzanthracene, a known carcinogenic compound in these animals. Lard was used as the suspending agent to insure slow absorption. Identical adequate food and living conditions were maintained. Feeding experiments were also undertaken. Merck's sulfanilamide and Eastman Kodak 1:2:5:6 dibenzanthracene were used. The experiment was arranged as follows:

Group I. Twenty mice were injected on 2 occasions with 15 mg dibenzanthracene in lard. They received a regular diet. Eight of these animals (40%) developed spindle cell sarcomas at the site of injection and died in 150 to 200 days after the first injection.

Group II. Twenty mice were injected with 15 mg sulfanilamide in lard on 2 occasions. They received a regular diet. Two animals (10%) developed tumors at the site of injection and died at 222 and 261 days respectively. Six animals are still alive. The tumors were frankly invasive and at death grossly deformed the animal. In one instance the tumor invaded the muscles of the leg and in the other instance the tumor eroded through the body wall, ribs and at autopsy was observed to be densely adherent to the liver. Microscopic section revealed the tumors to be composed predominantly of spindle cells with many polymorphic and tumor giant cells. The tumors were not transplanted. This procedure is contemplated, however. No metastases were seen but tumor cells were seen invading the liver in the instance mentioned above. Numerous mitotic figures were seen.

Group III. Twenty mice were injected with 15 mg dibenzanthracene in lard on 2 occasions. They received a diet which contained 2½% sulfanilamide. All the animals died prior to 150 days. Two spindle cell sarcomas were noted at the injection site (10%) at this time.

Group IV. Twenty mice were simply fed a diet containing 2½%

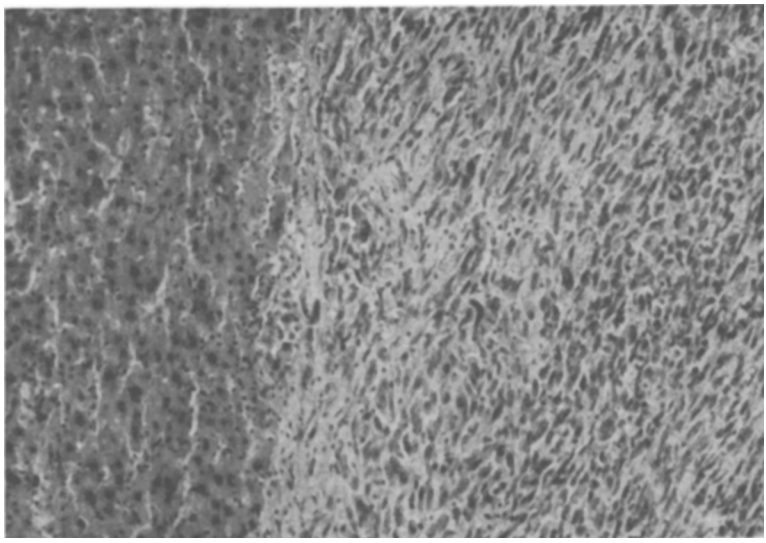

FIG. 1.

sulfanilamide. No injections were made. Most of the group is still alive. No tumors have been noted.

Group V. Twenty mice were fed a regular diet. No injections were given. Most of the group is still alive. No tumors have been noted.

In addition to the wide experience of numerous workers in the field of carcinogenic investigation which has pretty well established the fact that lard itself is not carcinogenic in numerous strains of mice,[4] the author cites the experience of Dr. A. Brunschwig who has been working with the above mentioned strain of mice in this laboratory at the same time as the author. The former worker obtained no tumors in Swiss albino mice following the injection of lard alone. This series contained 120 animals.[5]

Several additional observations were made in these experiments which will be reported at a later date. This work is being repeated and extended and a similar experiment is being carried out with sulfapyridine.

Summary. The subcutaneous injection of Merck's sulfanilamide suspended in lard into 20 Swiss Albino mice was followed in 2 instances by the development of a spindle cell sarcoma at the site of

4 Andervont, H. B., *Public Health Lib., U. S. Treas. Dept.*, 1934, **49**, 620.

5 Personal communication.

injection. The animals died at 222 and 261 days respectively. A similar group of mice treated similarly with 1:2:5:6 dibenzanthracene resulted in spindle cell sarcomas at the site of injection in 8 animals. The latter died in 150 to 200 days.

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Effect of Elementary Phosphorus Upon the Epiphysis of the Albino Rat.*

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(Introduced by L. Dienes.)

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Wegner described the development of a sclerotic zone in the vicinity of growing epiphyseal cartilage following the administration of small amounts of elementary phosphorus.[1] Since the process was subsequently found to be opaque to roentgen rays[2, 3, 4] it occurred to one of us (J.S.B.) that this phosphorus line might offer an *in vivo* radiographic marker of the varied growth rate in the epiphyseal region. No clear conception of the developmental mechanism of the line was available and this study was therefore performed in order to investigate its histogenesis.

Material and Methods. Subjects were albino rats 4 to 8 months of age sustained upon the BMS regimen recommended by Russell.[5] There were 28 experimental animals, 6 of which were utilized as controls. To the remainder a daily dose of 2.0 cc of elementary phosphorus in oil of peach kernels was administered by gavage. The solution contained 0.05 mg of elementary yellow phosphorus per cubic centimeter. Roentgenograms were taken of the limbs at intervals in order to detect the development of the opaque area of osteosclerosis.

Animals were sacrificed at periods up to 68 days after the initial administration of phosphorus. Both femurs and tibias were sub-

* The expenses of this study were defrayed by a grant from the Infantile Paralysis Research Fund of the National Foundation for Infantile Paralysis, Inc.

1 Wegner, G., *Virch. Arch.*, 1872, **55**, 11.

2 Park, E. A., Jackson, D., Goodwin, T. C., and Kadji, L., *J. Ped.*, 1933, **3**, 265.

3 Phemister, D. B., *J. A. M. A.*, 1918, **70**, 1737.

4 Phemister, D. B., Miller, E. M., and Bonar, B. E., *J. A. M. A.*, 1921, **76**, 850.

5 Russell, W. C., *J. Nutrition*, 1932, **5**, 347.