"glucocorticoids" presumably causes an increased utilization of amino acids which is associated either with inhibition of protein anabolism or with enhancement of protein catabolism. Consequently, an excessive demand for pyridoxine may be expected to result from the administration of 11-oxygenated adrenal steroids or of substances with similar activity. Conversely, androgenic steroids having a metabolic effect opposite to that of "glucocorticoids" may exert a sparing action upon vitamin B_6. Evidence supporting these possibilities has been obtained from the present experiments. Furthermore, both cortisone and testosterone were found to accentuate the retardation of lymphosarcoma growth associated with lack of pyridoxine. This effect in the case of testosterone was accompanied by an alleviation of the nutritional disease.

Summary. Marked retardation of growth of lymphosarcoma implants was observed in pyridoxine-deficient rats injected with 2 mg daily of cortisone or of methyl testosterone. Survival of pyridoxine-deficient, tumor-bearing rats was prolonged by testosterone and shortened by cortisone.

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Studies on the Early Phase of Induced Fibrosarcoma in the Hamster. (18054)

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We have been able to produce fibrosarcomas in the golden hamster by injection of 20-methylcholanthrene in tricaprylin or olive oil. Similar sarcomas have been produced in the same species by the injection of 9,10-dimethyl-1,2 benzanthracene(1). The incidence of tumors was high, and the true malignant nature of the new growths was indicated by their morphology and transplantability(1). The sarcomas induced by 20-methyl-cholanthrene were similar to those reported with benzanthracene; it was noted, however, that often they originated as early as the 60th day. This rapid induction was a favorable condition in which the earliest stages of sarcoma inception might be studied.

Experimental. Tumors were produced in 6 hamsters by the injection subcutaneously into the right median thigh area of 0.5 mg of 20-methylcholanthrene in 0.25 ml of tricaprylin. As soon as the first tumor appeared, trocar transplants were made in 7 animals. Thirteen days later all of the injected animals showed small tumors. Transplants were carried through 5 generations. Twelve animals were used in each generation of transplants. In the first generation, 11 takes were recorded; 10 in the second generation, 7 in the third, 9 in the fourth and 9 in the fifth. Growth of the transplants in the last series was evident within one week following transplantation.

Following this pilot experiment, groups of animals were injected subcutaneously with 1 mg 20-methylcholanthrene in: (1) olive oil plus carbon black, (2) saline suspension plus carbon black, (3) saline suspension. Twenty animals were injected in each group. In Group I, there were 19 animals which subsequently showed tumors. In Groups II and III, no tumors were found. Following in-

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1. Crabb, Edward D., *Cancer Research*, 1946, v6, 627.

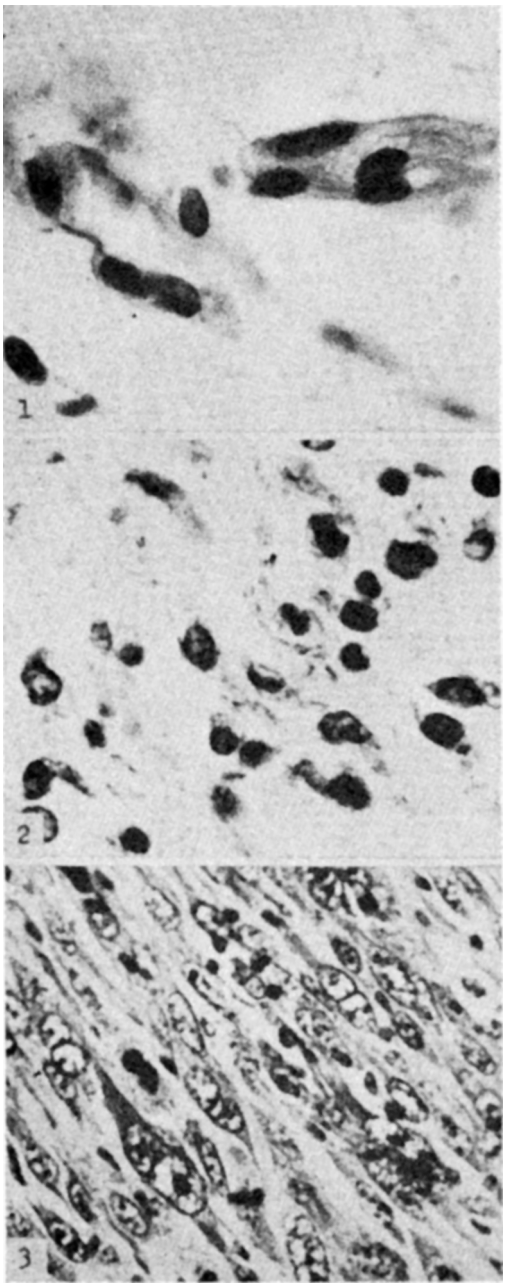

FIG. 1.

This photomicrograph of a 48 hour injection site shows 4 cells characteristic of the alteration which was first found at 24 hours and which persists until frank sarcoma supervenes. Note the massive edema spaces, blunt ended cells on right and tailed ones on left. The cytoplasm is swollen, hyperchromatic. The nuclei are very hyperchromatic. All sections —H. & E. stain.

FIG. 2.

A 4 day injection site showing connective tissue culture like arrangement of fibroblasts. Tailed and blunt cells and a few inflammatory cells are seen. Edema is still marked. H. & E. stain.

FIG. 3.

High magnification of one of the fibrosarcomas. Compare the large nucleated cells to the cell described in Fig. 2. The edema has disappeared and sheets of cells are seen.

jection animals were sacrificed in each group at 24, 48 hours; 3, 4, 7, 10 days and at 2, 3, and 4 weeks.

In the early part of the experiments, before neoplasia was apparent, tissue was removed from the site of the carbon black. Grossly, except for edema, there was little change at the injection sites. Small oil droplets of the injected solutions were found and after 3 weeks small areas of fibrosis could be discerned. Microscopically, however, striking tissue changes were found. Within 24 hours there was edema of the connective tissue, and within 48 hours these cells showed cytoplasmic granularity. The connective tissue cells, in the areas of injection, showed an alteration which was to remain constant in all sections studied until frank sarcoma was established. This alteration consisted of swelling and granularity of the cytoplasm with hyperchromatism. The nuclei were enlarged, very hyperchromatic, and either solid blue or very hydropic (Fig. 1 and 2). There was a marked edema which persisted until the end of the first week. A scattering of inflammatory cells were present, but there was little cell necrosis. The altered connective tissue cells were separated into single cells or small groups and were blunt ended or had long cytoplasmic tails. The groups of changed connective tissue cells were studied from the standpoint of their origin. They simulate macrophages but never, at any stage, did they contain carbon black. The capillary endothelial cells were not affected. In fact, the only cells which showed striking and constant cytoplasmic and nuclear alteration were the connective tissue cells. These cells within a brief period of 4 days were already forming into small sheets which simulated minute tissue cultures. After one week, the areas condensed into small nodules, which lay next to

the oil droplets. In these areas, macrophages filled with carbon black were readily distinguishable from sheets of connective tissue cells. Cell necrosis was never prominent following oil injections. In contrast, the areas in which 20-methylcholanthrene was injected in saline suspension showed early severe cell necrosis. At 3 days there was necrosis of connective and adipose tissues with cell alterations similar to, but not as intense as, those seen surrounding fat solutions. Many tissue slits were found in which the crystals had been dissolved and giant cell formation was found. The necrosis became more extensive up to one week, and by ten days the process appeared static. In oil injection, poor granulation tissue formed, and by the third week, microscopically large sheets of abnormal connective tissue cells have formed. Within 8 to 10 weeks from these sites frank sarcomas grew (Fig. 3). As nearly as can be ascertained from studying sections from different animals in the series at the sites there are stages of cell proliferation followed by regression. The whole cycle is repeated a number of times before the true infiltrative sarcoma is finally established.

Discussion. Most investigations on tumor induction have studied the tumor after it became established. By marking the site with carbon black, we were able to study the cell alterations at an extremely early time. With this technic it became clear that carcinogen produced a distinctive cell alteration which is established within 72 hours following the injection and that this alteration was neither extensively destructive to the cell nor directly stimulatory to cell growth. Wolbach(2) in studies on skin arrived at a similar conclusion for that tissue. The changes induced *in vivo* are very similar to those that had previously been found to occur when connective tissue cultures are exposed to a carcinogen(3). It was surprising to find this distinctive cell alteration taking place so long before invasive sarcoma finally is found. Wolbach(4) further found that the carcinogens kept the tissue from healing. There was a cut down in reparative processes. We noticed a similar finding and the same kind of phenomenon has been noted in tissue cultures by Voegtlin(5), who found a striking growth inhibition in methylcholanthrene exposed cultures.

A variety of special stains were done on the established tumors in order to try to specify their origin. The results of these special stains were often equivocal. With the technic of marking the macrophages with carbon black and studying the other tissue components, it was clear that from the beginning the cells which finally grew into the fibrosarcoma originated from the connective tissue fibroblasts. Orr(6) who did a study of tissues removed somewhat later than ours noticed that formation of fibrous connective tissue was deficient at the site of carcinogen injection, but did not study the cell race of origin.

The edema which is noted during the first week is excessive and finally disappears; this is out of proportion to the very scant amount of inflammatory exudate in the lipoid injected sites. Following the edema phase, the altered connective tissue cells grow in a small locus in fairly close proximity to the injected areas. The lack of cell necrosis would seem to rule it out as an essential characteristic of the induction process.

Summary. The subcutaneous injection of 20-methylcholanthrene into hamsters produced fibrosarcomas within 60 to 80 days. Examination of the injection sites, 24 hours following injection, showed fibroblasts which are morphologically different from normal. At first these cells show little active proliferation. The altered fibroblasts form a distinct strain of cells in a nodulus close to the area of injection and it is from these nodules of altered fibroblasts that invasive fibrosarcoma grows. In contrast to the oil solutions of carcinogen used, the saline suspensions produce cell necrosis and giant cell reaction.

2. Wolbach, S. B., *Am. J. Path.*, 1937, v13, 662.
3. Earle, W. R., and Nettleship, A., *J. Nat. Cancer Inst.*, 1943, v4, 213.
4. Wolbach, S. B., *Arch. Path.*, 1936, v22, 279.
5. Earle, W. R., and Voegtlin, C., *Am. J. Cancer*, 1936, v34, 373.
6. Orr, J. W., *J. Path. and Bact.*, 1939, v49, 157.

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