adrenal weight increase following ACTH administration. A number of workers(13-15) have reported a lack of correlation in the biological activities of various ACTH preparations. These findings have led to the recent viewpoint(15) that there are several adrenocorticotropic hormones having different biological activities. Until this question is resolved, conclusions are not justified concerning the pituitary or adrenal origin of the impairment in adrenal cholesterol depletion observed in the pyridoxine-deficient rat. One may not at present regard the depletion of ascorbic acid and cholesterol of the adrenal and its weight increase as comparable biological activities measuring the same functional activity of the gland. Better understanding is needed of the physiological processes involved in the production of adrenal cholesterol and its relationship to hormone production.

Summary. Two days after hypophysectomy, the pyridoxine-deficient rat treated with ACTH had the same amount of adrenal ascorbic acid depletion and adrenal weight increase as did the pair weighed and *ad libitum* controls but like these controls no decrease in adrenal cholesterol. When intact animals were subjected to hypoxia, however, the pyridoxine-deficient group, although able to maintain adrenal weights and adrenal ascorbic acid content as did the pair weighed and *ad libitum* groups, clearly failed to show the adrenal cholesterol depletion characteristic of the latter groups. The depletion of adrenal cholesterol is apparently not controlled by the same mechanism involved in adrenal hypertrophy and adrenal ascorbic acid depletion.

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Carcinogenic Activity of Radioactive Phosphorus.* (21070)

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The development of malignant tumors has been described in rats which survived a dose of radioactive phosphorus in the LD_{50} range, *i.e.,* 4.5 μc per gram body weight(1). The present study indicates that even when substantially smaller doses are used, P^{32} is still a potent carcinogenic agent.

Method. A total of 123 adult white male rats of the Wistar strain, weighing about 150 g each, obtained from Carworth Farms, were used. These were divided into 6 groups in which each animal received a single dose of 0.25, 0.5, 1.0, 2.0, 3.0 or 3.5 μc of P^{32} per

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TABLE I. Incidence of Malignant Tumors.

Dose of P^{32} (μc/g body wt)	Original No. of rats	Rats alive after 8 mo: No.	Rats alive after 8 mo: Life span (mo)	Rats alive after 8 mo: % incidence of malignant tumors
.25	25	21	11–35	0
.5	25	22	11–31	0
1.0	20	19	13–24	32
2.0	16	13	11–30	31
3.0	20	18	9–32	22
3.5	17	16	9–12	50

gram body weight respectively. The rats were housed individually in an air-conditioned unit permitting constant temperature and humidity. Radioactive material was administered by the intraperitoneal route. After 8 months periodic x-rays were taken of the entire skeleton of most animals and a routine x-ray of the skeleton was made of every rat at the time of death.

Results. Table I shows the number of animals in each group originally, the number alive after 8 months and their subsequent life span, and the incidence of malignant neoplasms. In calculating incidence those rats in the 0.25, 0.5, 1.0 and 2.0 μc groups which died before 8 months and those in the 3.0 and 3.5 μc groups which died before 5 months, *i.e.*, considerably before the latent period for tumor development, were not included. Also lymphosarcoma, which occurred in one animal in each of the 0.25, 1, 2 and 3.5 μc groups was omitted since this tumor has occasionally been observed to occur spontaneously in the C. F. Wistar strain.

Outside of one rat with lymphosarcoma, the 43 animals receiving the 0.25 or 0.5 μc dose of P^{32} did not develop tumors. In the groups where dosage ranged from 1.0 to 3.5 μc of P^{32}, neoplasms occurred in from 22 to 50% of animals. The greatest frequency was in rats which received the highest dose of radioactive phosphorus. However, among the 1, 2 and 3 μc groups there was no indication that the incidence of malignancy decreased with dosage in the manner described for radioactive strontium(2).

Table II gives the number of rats in each group with neoplasm, the latent period for tumor development, and the types of neoplasm. Only 2 types were observed, osteogenic sarcoma and squamous cell carcinoma. The former was more common and occurred in a total of 18 animals with the following locations: femur 7, spine 4, tibia 3, jaw 3, and humerus 1. There were metastases in 10 of the 18 rats, usually to lungs and regional lymph nodes. Six animals developed an extensive ulcerating squamous cell carcinoma involving one side of the face with no metastasis. Except for one rat injected with 1 μc of P^{32} per gram body weight, the squamous cell tumors occurred in animals which received the highest dose of radioactive phosphorus, *i.e.*, 3.5 μc per gram body weight.

Discussion. The overall incidence of neoplasms produced by smaller amounts of P^{32} was less than that previously obtained with very high doses of the isotope(1). In addition the latent period of tumor development appeared to be lengthened when the dose of P^{32} was lowered. Thus the mean time of development was 19, 14 and 10.5 months respectively for animals given 1, 2 and 3 μc of P^{32} per gram body weight. This observation is limited by the fact that these periods represent detection of the neoplasm rather than the exact time of origin.

The two types of malignant tumor induced in this study were the same as those which resulted from large doses of P^{32}. Osteogenic sarcoma was the most common neoplasm. This would be anticipated since P^{32} is deposited heavily in the skeleton and causes radiation osteitis which is a precursor to malignant change. The second variety of neoplasm resulting from P^{32} was squamous cell carcinoma of the face. This appeared to arise below the skin rather than from the surface and to invade the subcutaneous soft tissues, bones of the face and orbit. While the exact origin has not been determined, the probable source is epithelium closely approximated to bone, *i.e.*, lining of accessory nasal sinuses, nasopharynx, and roof of mouth, and presumably irradiated from this site.

The validity of our data obviously depends on the spontaneous incidence in rats of osteogenic sarcoma and cancer of the face. As far as is known, these tumors are rare among normal animals of the Wistar strain. In 58 such rats followed through their lifetime, we

TABLE II. Latent Period and Types of Neoplasm.

Dose of P^{32} (μc/g body wt)	No. of rats with neoplasm	Latent period (mo) Range	Mean	Types of neoplasm: Osteogenic sarcoma	Types of neoplasm: Squamous cell carcinoma
1.0	6	12 – 25	19	5	1
2.0	4	12 – 15	14	4	0
3.0	4	8 – 14	10.5	4	0
3.5	8	7.5 – 13	10	5*	5*

* Two rats in the 3.5 μc group had both osteogenic sarcoma and squamous cell carcinoma.

found one instance of osteogenic sarcoma and none of squamous cell carcinoma(3). Ratcliffe(4) reported that about 5% of rats in a Wistar stock colony developed neoplasm and that among the 75 tumor-bearing animals comprising this 5% there was one osteogenic sarcoma and one carcinoma of the skin. The latter arose from the tip of the snout. A very low overall incidence of these two malignant neoplasms has also been described in other strains of the rat(4,5).

In this study the smallest dose of P^{32} which induced tumors was 1 μc/gram body weight and this corresponds to 70 mc for a 70 kg human, *i.e.*, about 5 times the usual therapeutic dose. Amounts of 0.5 and 0.25 μc of P^{32} per gram, which approach the average therapeutic dose in man, did not prove carcinogenic. From the experimental standpoint P^{32} can be used as an effective and relatively simple method of inducing malignant neoplasms. The optimum dosage is probably from 1 to 3 μc per gram body weight. Larger amounts will also be carcinogenic but the early mortality from radiation is potentially greater and the life span of the surviving animals shorter.

Summary. 1. Single doses of radioactive phosphorus, administered internally to rats in amounts of from 1 to 3.5 μc/gram body weight, proved carcinogenic. Two types of malignant neoplasms were produced, *i.e.*, osteogenic sarcoma and carcinoma of the face. 2. The incidence of neoplasms ranged from 22 to 50%. The latent period of tumor development was lengthened with smaller doses of P^{32}. 3. Amounts of P^{32} less than 1 μc/gram body weight failed to induce neoplasms.

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Chemical Inhibition of Cell Division of *Escherichia coli* by Diazo Compounds and Antagonism by Tyrosine. (21071)

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Studies with *Escherichia coli* have shown that certain organic chemicals, such as nitrogen mustards, triethylenemelamine, diazouracil, and other diazo compounds produce the same morphological effects, *i.e.*, the inhibition of multiplication without inhibition of culture growth or mass, as alpha or X-irradiation(1,2) and produce similar biochemical transformations(3). Since 5-diazouracil had proved to be a highly effective cell-division inhibitor and since both 5-aminouracil and

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