religious and scientific creeds, to the lovers of all our different schools of poetry and art, ancient and modern, and to many more besides these. It is the highest common denominator in the spiritual life of to-day.

Let us hope that all the American Republics will sign, ratify and pass enabling laws to get the greatest benefit from this International Convention as soon as possible. We also hope that our Government, which stands first in conservation measures in the Americas, will lead the way by being the first to ratify.

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# SPECIAL ARTICLES

## UPTAKE OF RADIOACTIVE PHOSPHORUS BY NUCLEI OF LIVER AND TUMORS

MICE carrying 10- to 15-day lymphoma[1] transplants were given 0.1 cc of isotonic $Na_2HPO_4$ containing 6–10 microcuries of $P_{32}$ by intravenous injection. At intervals afterwards the livers were cleared of blood by perfusion with saline, immersed in ice cold 5 per cent. citric acid for one-half hour, and the nuclei isolated by centrifugation of the pulped tissues. Tumors were removed from the same animals and similarly treated to obtain nuclei. Haemacytometer counts were made on all samples of nuclei, and contamination by fragments of cytoplasm was found to be negligible.

Fig. 1 shows the microcuries of $P_{32}$ per cm$^3$ packed

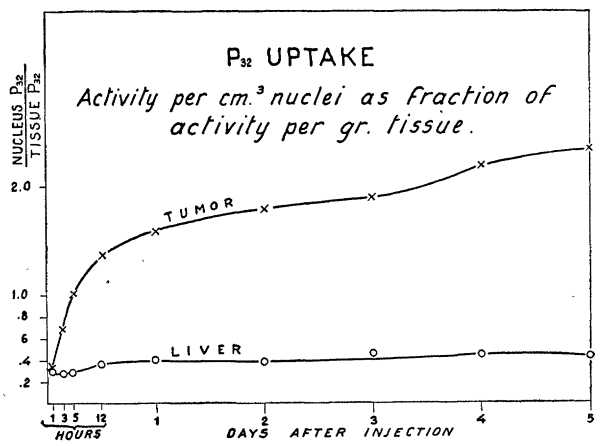


FIG. 1.

nuclei as a fraction of the $P_{32}$ activity per gram wet weight of whole tissue plotted against time after injection. Whereas the fraction of the liver $P_{32}$ bound by the nuclei remains constantly at .3–.4, the relative concentration in the tumor nuclei rises to more than twice that of the tumor tissue. Similar results have been obtained with sarcoma 180 in the mouse.

Isolated nuclei suspended in isotonic $Na_2HPO_4$ containing $P_{32}$ take up only one tenth the *in vivo* nuclear activity, and most of the *in vitro* activity can be shown to be adsorbed phosphate. Nuclei from liver slices shaken at room temperature in isotonic $Na_2HPO_4$ have less than one hundredth of the *in vivo* concentration of $P_{32}$. The uptake of $P_{32}$ can not therefore be attributed to simple chemical exchange.

After extraction with alcohol-ether and trichloracetic acid 60 to 70 per cent. of the $P_{32}$ of the liver nuclei is in the residue (nucleoprotein) and 70 to 95 per cent. of the $P_{32}$ of the tumor nuclei is in the same fraction at all intervals from one hour to five days after injection.[2] The data indicate that $P_{32}$ may be built into the nucleoprotein directly from the inorganic phosphate or that the rate of turnover in the more labile forms of organic phosphate is very rapid. Since the metabolic rate is lower in the rat than in the mouse, it is expected on the latter hypothesis that for comparable tissues (*e.g.*, liver) the relative $P_{32}$ concentration in the nuclei of the rat will be lower than for the mouse. From one-half hour to two days after injection of $P_{32}$ into 150-gram rats, the concentration in the liver nuclei is approximately 0.1 per cent. of the injected dose. The second alternative, therefore, seems the more probable.

To determine whether the greater concentration of $P_{32}$ by tumor nuclei is characteristic of the tumor *per se* or is to be attributed to mitotic activity, three 150-gram rats were partially hepatectomized and given $P_{32}$ intravenously 36 hours later. Three normal rats of the same weight, and three rats carrying bilateral carcinoma 256 implants were injected at the same time. Two days later livers and tumors were removed. The ratios of nuclear $P_{32}$ to tissue $P_{32}$ were .345, 1.02 and 1.08 for nuclei from normal liver, regenerating liver and tumor, respectively. With rats injected four days after partial hepatectomy and nuclei removed three days after $P_{32}$ injection, the ratios for nuclei from normal and hepatectomized animals were .28 and .32, respectively. Very few of these nuclei were found in mitosis, while of the liver nuclei removed in the first experiment 3.7 per cent. were in anaphase or metaphase and a much larger per cent. in prophase. The

[1] J. H. Lawrence and Gardner, *Am. Jour. Cancer*, 33: 112–119, 1938.

[2] The data are insufficient as yet for determining whether the difference in nucleoprotein $P_{32}$ of tumor and liver nuclei is significant. (71, 67, 64, 68, 53, 72 per cent. in liver nuclei at one hour, 1, 2, 3, 4, 7 days after injection and 98, 95, 94, 70 per cent. in lymphoma nuclei at 1, 3, 5, 7 days.) Preliminary data show no difference in nucleoprotein $P_{32}$ of rat liver and carcinoma nuclei.

greater $P_{32}$ uptake by tumor nuclei should therefore be attributed to mitotic activity.

There are $14.8 \times 10^{-10}$ mg P/liver nucleus and $12.8 \times 10^{-10}$ mg P/tumor nucleus. From the nuclear phosphorus composition and the nuclear $P_{32}$ content at 48 hours, the specific activity of tumor nuclei is found to be more than four times that of liver nuclei. Calculating from the daily $P_{31}$ retention[3] and the per cent. $P_{32}$ uptake, each nucleus will incorporate a quantity of $P_{31}$ equal to 8.9 per cent. of its $P_{31}$ content in five hours. It will thus take 56 hours for the nucleus to synthesize a quantity of $P_{31}$ equal to the amount it originally contained. This should be the time required to synthesize a new nucleus. This value agrees approximately with the observed rate of growth of the tumor which doubles in size in about two days at this phase of its growth curve.[4] It is interesting, though it may be purely a coincidence, that approximately the same time is obtained for the duration of the mitotic cycle in root tips by analysis of effects of x-rays and neutrons.[5]

A detailed account of these experiments will be presented elsewhere.

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## MODE OF ACTION OF ESTROGENS ON THE MAMMARY GLAND

FOLLOWING the demonstration by Lyons and Pencharz[1] in 1936 of the failure of estrogenic hormone to produce growth of the mammary glands of hypophysectomized guinea pigs, a sisable literature has appeared in confirmation of this work, which has been extended to include the rat, mouse, cat, rabbit and ground squirrel.[2] These observations emphasized the importance of the pituitary to the mammary gland and gave rise to the theory that injected estrogens exert their effects through the mediation of the pituitary, which was postulated to produce a specific mammogenic hormone.[3]

The specificity of the relationship between the pituitary and mammary growth has, however, been called into serious question by the demonstration[4] that in intact rats which were restricted to a diet comparable to that consumed by hypophysectomized litter-mates, the glands likewise failed to respond to administered estrogen. It has been shown,[5] further, that when hypophysectomized rats were treated with growth complex, growth of the mammary gland could be elicited by estradiol benzoate, and that the degree of stimulation could be correlated with the weight gain of the animals.

Recent experiments permit of a new approach to the problem of the mode of action of estrogenic substances on the mammary gland. By the direct application of the hormone to the nipple area it is possible to obtain a local effect on the nipple and mammary gland. In this manner one can observe the effects of the test substance in the intact animal, thereby obviating the complication of nutritional disturbances produced by hypophysectomy.

Immature male rhesus monkeys were used. The left nipple of each animal was painted daily with a solution of estrone in 95 per cent. alcohol, 0.05 mgm per cc. To the right nipple alcohol alone was similarly applied. Two animals were so treated for a period of 75 days. The entire glands were then removed. A third animal was treated in this manner for 50 days, and the breasts were removed 105 days later. The mammary glands were then fixed, dissected, stained and cleared, and studied as whole mounts.

In all cases the left mammary gland was distinctly larger and more developed than the right one (see Fig. 1). This difference in size exceeded, in each instance, the minor variations between right and left glands occasionally encountered in a study of more than 200 pairs of control monkey mammaries, including the breasts of 30 males.

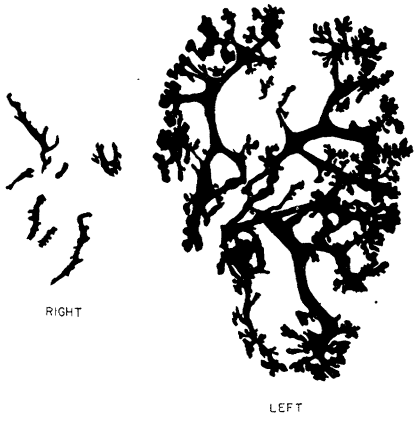


FIG. 1. Right and left mammary glands of prepubertal male monkey after daily application of alcoholic solution of estrone to left nipple for 75 days. Silhouette tracings from photographs of actual specimens.

These observations are regarded as evidence for the direct action of percutaneously administered estrogen on the mammary gland. While not controverting the possible existence of pituitary mammogenic hormones,

[3] L. W. Tuttle, personal communication.

[4] Growth curves obtained from I. L. Chaikoff and H. B. Jones, unpublished.

[5] A. Marshak, *Proc. Nat. Acad. Sci.*, 25: 502–510, 1939.

[6] Fellow of the John Simon Guggenheim Memorial Foundation.

[1] W. R. Lyons and R. I. Pencharz, *Proc. Soc. Exp. Biol. and Med.*, 33: 589, 1936.

[2] E. T. Gomez and C. W. Turner, *Mo. Agr. Exp. Sta. Res. Bul.* 259, 1937.

[3] A. A. Lewis and C. W. Turner, *Mo. Agr. Exp. Sta. Res. Bul.* 310, 1939.

[4] E. B. Astwood, C. F. Geschickter and E. O. Rausch, *Am. Jour. Anat.*, 61: 373, 1937.

[5] I. T. Nathanson, D. T. Shaw and C. C. Franseen, *Proc. Soc. Exp. Biol. and Med.*, 42: 652, 1939.