Some Factors Influencing the Total Phosphorus Content of Rat Uteri. (18861)

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In the course of studies of the uptake of P^{32} by uteri of the albino rat(1), it was observed that a straight line relationship existed in certain instances between total phosphorus and wet weights of the uteri. There were no data available in the literature on this relationship. Studies were therefore outlined to extend these observations under varying conditions. These were to include the effect of estradiol, of hypophysectomy and of age on the phosphorus content of the rat's uterus as it related to the weight of the organ.

Materials and methods. Female albino rats of the Sherman and Wistar strain were used. Their diet was unrestricted and consisted of dog chow pellets, occasional bread and lettuce, and water. The animals comprised three groups: (1) immature (21-26 days old); (2) adolescent (approximately 2 months old); and (3) adult (3 to 7 months old). Hypophysectomy was done by the parapharyngeal route. If completeness of hypophysectomy was doubtful in any instance, the animal was not included in the study. Estradiol was injected subcutaneously in sesame oil or in dilute (20%) alcoholic solution. Two or 3 daily injections were given with doses ranging from 0.11 γ to 1.0 γ. Twenty-four hours after the last injection the animals were killed with chloroform, and the uteri were quickly excised and weighed. In some instances the uteri were expressed between filter paper. The uteri were each digested in 50 ml beakers with 5 ml of concentrated nitric acid on a hot plate at low heat. When almost dry, they were allowed to cool and 1½-2 ml of concentrated hydrochloric acid were added. After intermittent gentle heating until gas evolution ceased, they were taken almost to dryness, when 1½-2 ml water were added and the volume was again reduced to a few tenths of a milliliter. The phosphorus was then determined on a suitable aliquot by the method of Fiske and Subbarow (2), using an Evelyn Photoelectric Colorimeter with 660 filter. In taking up the contents of the beakers for analysis a small amount of carbon tetrachloride was added to

TABLE I. Data Regarding Groups of Animals and Manner of Treatment.

No. of rats	Age in days	Pituitary	Days after hypophysectomy	Uterus expressed	Treatment
10	21–26	Intact		No	None
8				"	Estradiol in oil .11–.21 γ daily for 2 days
5				Yes	None
3				"	Estradiol in oil .2 γ daily for 3 days
4	60–80			No	None
4	94–207			"	"
15				Yes	"
10				"	1 γ estradiol in oil daily for 3 days
8	21–26	Removed	7	"	Estradiol in oil .3–.4 γ daily for 3 days
4			7	"	None
2	94–207		39	No	"
8			6–55	Yes	"
8			24–146	"	Estradiol in oil 1 γ daily for 3 days
8			8–59	"	Estradiol in 20% alcohol .25–.5 γ daily for 3 days

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1. Grauer, Robert C., Strickler, Herbert S., Wolken, Jerome J., and Cutuly, Eugene, PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 651.

2. Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, v66, 375.

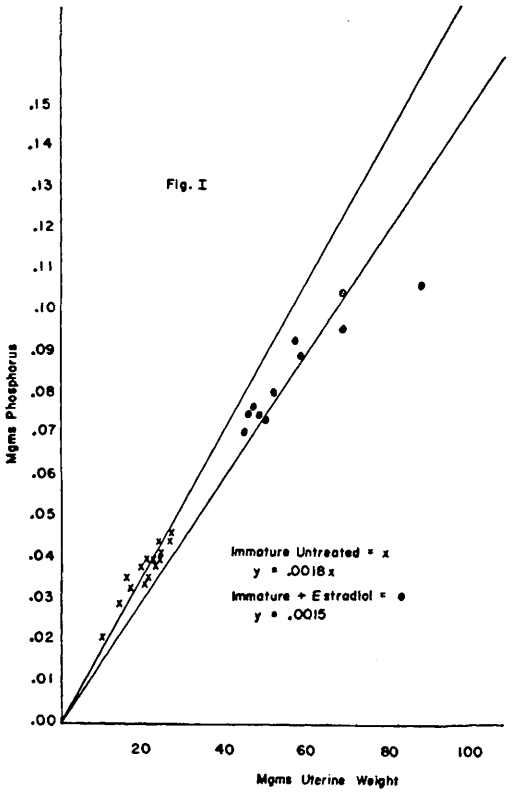

FIG. 1.

prevent occlusion by any residual fatty acid material. Since plots of the 8 sets of experimental data indicated the relationship to be of the general linear form $y = b + mx$, it was decided to evaluate the constants of the equation pertinent to each group of data. The boundary condition $b = o$ was applied; it was therefore necessary to compute "m" only. This was done using the method of least squares.

Results. Table I shows the numbers of rats, the ages in days, whether or not the pituitary had been removed and if so the time that elapsed, whether or not the uteri were expressed and finally, the treatment if any with estradiol.

The 4 figures show mg total phosphorus plotted against uterine weight in mg for the various groups. Fig. 1 shows the two curves $y = .0015x$ and $y = .0018x$ for the immature animals with and without estradiol respectively. There is a somewhat lower phosphorus content per mg of uterine weight in the estradiol treated animal than in the untreated animal. Fig. 2 shows the curves $y = .0024x$ and $y = .0015x$ for a small group of immature hypophysectomized animals without treatment and for a larger group treated with estradiol, respectively. Here the phosphorus content per unit weight of the uterus of the immature hypophysectomized untreated rats is significantly higher than those treated with estradiol. The curve for the estradiol treated hypophysectomized immature animals (Fig. 2) is identical in slope with that for the estradiol treated intact immature animals (Fig. 1). Fig. 3 shows the curves $y = .0016x$ and $y = .0017x$ for adult hypophysectomized rats with and without estradiol. The phosphorus content per mg of uterus is practically identical in these 2 groups. These slopes also are quite near the curve of the estradiol treated immature rats (Fig. 1). Fig. 4 shows the curve of the lowest slope, $y = .0011x$ with considerable scatter in the younger mature untreated intact animals. The equation for the adult intact estradiol treated animals is virtually identical, being $y = .0012x$.

Discussion. These data have been ex-

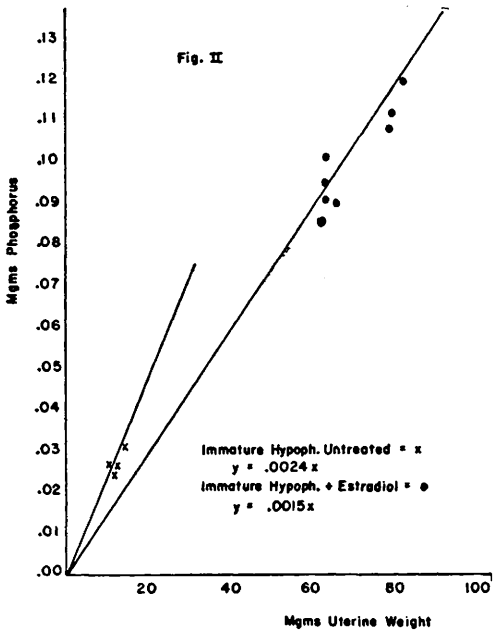

FIG. 2.

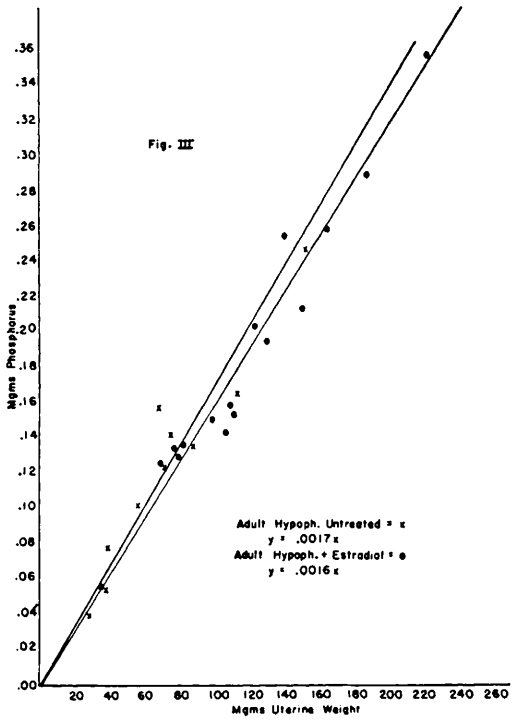

FIG. 3.

pressed in terms of simple straight line functions, $y = mx$, where m is milligrams of phosphorus per mg of wet weight of uterus. On this basis it was observed that the highest concentration of phosphorus occurred in the uteri of the immature hypophysectomized untreated rats. Conversely, the adult untreated rats and the adult estradiol treated rats showed the lowest phosphorus content per mg of uterine tissue (Fig. 4). Such relationships of phosphorus turnover to phosphorus content of the cells may be governed by hormonal factors under the influence of the pituitary gland. In this connection it may be pointed out that Gemzell and Samuels(3) observed that the rate of turnover of inorganic phosphorus in the rat was markedly reduced by hypophysectomy in all the tissues examined, but that it did not affect the concentration of the phosphorus.

The two factors to be considered in evaluating our observations are those of hypophysectomy and of the effect of estradiol on the phosphorus content of the uterus. Hypophysectomy causes reduction in metabolic processes in all of the tissues with a consequent retardation in turnover of the phosphorus in the uterus. The concentration of phosphorus in the immature untreated animal (Fig. 1) was highest after hypophysectomy (Fig. 2). This may represent gradations of the phosphorus content of the target organ (uterus) mediated through the stimulation of the ovaries by the pituitary. As the adult state of equilibrium is approached by the animals and the ovaries are producing endogenous estradiol, the phosphorus content per mg of uterine tissue decreases. The injection of exogenous estradiol in the adult rats did not appreciably alter this ($y = .0011x$ and $y = .0012x$). Reference to Table II indicates these trends. The data were arranged in 2 groups; group A represents those animals that were untreated and group B includes those which received estradiol. In both groups age and/or hypophysectomy were the variables. The magnitude of the dose of estradiol was not an important factor.

The adult hypophysectomized animals and the immature intact animals had practically the same phosphorus concentrations in their uteri per unit weight of organ when they were treated with estradiol. Estradiol presumably causes an increased rate of phosphorus turn-

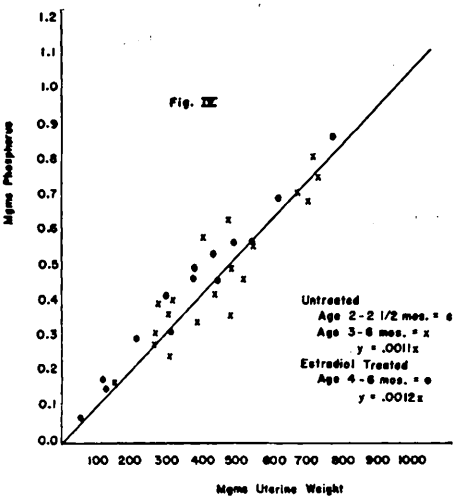

FIG. 4.

3. Gemzell, Carl A., and Samuels, Leo T., *Endocrinology*, 1950, v47, 48.

TABLE II. Influence of Hypophysectomy and of Estradiol on Phosphorus Content of the Uterus.

Group A—Untreated	
Adult intact	$y = .0011x$
" hypoph.	$y = .0017x$
Immature intact	$y = .0018x$
" hypoph.	$y = .0024x$
Group B—Estradiol treated	
Adult intact + Est.	$y = .0012x$
" hypoph. + Est.	$y = .0016x$
Immature hypoph. + Est.	$y = .0015x$
" intact + Est.	$y = .0015x$

over in the uterus and overcomes the retarding influence of hypophysectomy on this target organ. It acts as an "equalizer" with respect to the phosphorus in the uterus of the immature intact, immature hypophysectomized, and adult hypophysectomized rat.

Investigations conducted in this laboratory showed the total phosphorus in the liver in both the immature and adult rat(4) to be the same per unit weight of tissue. The reason that the uterus does not act in this manner in the immature untreated rat as compared to the uterus of the adult rat may be explained by the fact that the uterus is a target organ, subject to changing metabolic processes with age. As maturity is approached the ovarian hormones, such as estradiol, may stimulate the phosphorus turnover and bring about an altered phosphorus content in the adult rat uterus.

From these observations it would appear justifiable to postulate that ovarian hormones, produced by way of pituitary activity, are important factors responsible for gradations of the phosphorus content of the uterus in varying conditions of age and glandular activity.

Conclusions. 1. The phosphorus content reaches a maximum per mg of uterine tissue in the immature untreated hypophysectomized animal. 2. The phosphorus content per mg uterine tissue is lowest in the adult and adolescent group in both the untreated and estradiol treated rats. 3. The phosphorus content per mg uterine tissue of estradiol treated animals is less than in the untreated animals. 4. Estradiol increases uterine weight and presumably stimulates phosphorus turnover but it does not increase the phosphorus content of the uterus. 5. The importance of estrogenic and pituitary hormones with respect to phosphorus metabolism in the uterus of the rat has been emphasized.

4. Strickler, Herbert S., Cutuly, Eugene, Grauer, Robert C., and Wolken, Jerome J.; Unpublished data.

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Evidence of Hypothalamic Control of the Pituitary Release of Thyrotrophin.* (18862)

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Although it has been postulated for many years that the hypothalamus in some manner regulates the secretion of thyrotrophin by the hypophysis, clear-cut experimental evidence demonstrating this phenomenon has not yet been presented(1). The present investigation was designed to provide a maximal stimulus for thyrotrophin release by feeding animals with hypothalamic lesions a diet containing propylthiouracil. In addition to thyroid size and histologic evidence of activity the iodide-concentrating capacity of the thyroid glands was determined, since this had previously been shown to be the most sensitive index of endo-

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1. Harris, G. W., *Physiol. Rev.*, 1950, v28, 139.