TABLE IV. Toxicity of Aureomycin and Chloramphenicol for Embryonated Eggs.

Dose in μg	Route	Aureomycin	Chloramphenicol
1000	Yolk sac	8/24*	2/21
800	Chorioallantois	17/17	
200	"	6/12	2/18

* Numerator: No. of embryos dead within 24 hr after treatment. Denominator: Total No. of eggs injected.

ference in toxicity between different lots. We also found our aureomycin preparation to be toxic for the chick embryo. As can be seen from Table IV, all of the embryos which were treated with 800 μg of aureomycin per egg by the chorioallantoic route died, while most of the embryos tolerated 1,000 μg or 2,000 μg per egg given into the yolk sac. This finding might well be correlated with the observations made by Lépine and his associates(10) that the growth of certain proliferating cells was definitely retarded by 100 μg of aureomycin and chloramphenicol per ml of tissue culture medium, and completely inhibited by 1,000 μg per ml. Moreover, Womack and his associates(11) demonstrated the presence in egg yolk of a substance which inhibits deterioration of aureomycin activity. The number of embryos treated was too small to evaluate the relative toxicity of aureomycin and chloramphenicol for the chick embryo, although aureomycin seemed to be a little more toxic than chloramphenicol.

Conclusion. A study of aureomycin, chloramphenicol and para-aminobenzoic acid in myxoma virus and vaccinia virus infection in the chick embryo failed to show any chemotherapeutic or chemoprophylactic action of these agents, either when they were used alone or in combination. In agreement with others, we also found that aureomycin, when introduced onto the chorioallantois, appeared to be toxic for the chick embryo.

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Influence of Estradiol on P^{32} Uptake by the Uterus. (18294)

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The object of this investigation was to secure increased concentration of a radioactive isotope in a target organ of the female genital tract. Evidence has been adduced that the weight response of target organs in treated animals may be influenced in an unexpected manner by varying the proportions of the constituents of the hormonal mixtures without changing the total equivalent doses(1,2). In order to elucidate the mechanism of such reactions experiments employing radioactive phosphorus (P^{32}) were designed. In the light of the observation that the rate at which new tissue is formed is one of the factors that influences the uptake of phosphorus(3), it seemed reasonable to assume that the uptake of P^{32} by a target organ, such as the

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TABLE I. Effect of Single Injection of Aqueous Estradiol on P^{32} Uptake by Immature Uterus.

Total No. animals	Avg time in hr	Avg uterine wt in g	Stand. error*	% dose × 10³†	% dose/g‡	Stand. error	Inj.§
4	3	.02	±.005	19	0.92	±.17	P^{32}
10	3	.03	±.003	44	1.26	±.10	" + Est.
4	6	.02	±.002	22	0.95	±.22	"
8	6	.04	±.006	46	1.23	±.09	" + Est.
3	24	.03	±.003	27	1.01	±.26	"
9	24	.05	±.005	77	1.67	±.12	" + Est.
3	48	.02	±.001	15	0.81	±.11	"
8	48	.03	±.003	64	1.86	±.11	" + Est.
2	72	.02		14	0.83		"
9	72	.02	±.002	42	1.75	±.14	" + Est.

* Stand. error $= \sqrt{\frac{\Sigma\Delta^2/n-1}{n}}$.

† % dose $= \frac{\text{c/m sample}}{\text{c/m dose}} \times 100$.

‡ % dose/g $= \frac{\left(\frac{\text{c/m sample}}{\text{c/m dose}} \times 100\right)}{\text{g wt of organ (wet)}}$.

§ Dose employed = 0.3-0.42 γ estradiol 4.0 μc P^{32}.

uterus, might be enhanced if that organ were stimulated by estrogens.

Material and method. Female rats 19 to 23 days old were employed in this study. Our previous investigations(2) indicated that the body weight had no significant effect upon the uterine response to injected estrogens. All injections, both of P^{32} and of estrogens, were made subcutaneously. The first experiment (Table I) comprised a group of 60 animals which were divided into 2 groups; those which received estradiol plus P^{32} and, those which received P^{32} alone (injected controls). The estradiol was administered in 20% alcoholic solution in doses ranging from 0.3 to 0.42 γ of estradiol. It was found(2) that this dose of estradiol gave the maximum uterine weight increment response. These animals received simultaneously 4.0 μc of P^{32}. The injected control animals received 0.5 cc of a 20% alcoholic solution containing P^{32} only. The animals were killed with chloroform at 3, 6, 24, 48 and 72 hours after injection. The uteri were immediately dissected, excised and spread on tared radiation planchettes. They were promptly weighed on a chainomatic analytical balance with magnetic damping and then dried under an infra-red lamp. In most cases the uteri were counted using a thin (2 mg/cm²) mica window counter, but a few were counted with a glass tube. All counts were corrected for background and radioactive decay. Counts agreed before and after rotation of the planchettes through 90°.

A second group of animals (Table II) was employed in order to determine whether the radioactive phosphorus became bound in the cell and did not represent a water transport. Thirty-six animals were treated in the same manner as the aforementioned group. Two groups were employed and were designated as "run 1" and "run 2." For purposes of fractionating, the uteri were removed in pairs and placed in a tared homogenizer (double tube type). They were weighed and placed in a dry ice acetone bath. The uteri were then ground with emery and 1 ml of water. 1.0 to 1.2 ml of 10% trichloracetic acid (TCA) was added and the mixture was filtered through No. 50 Whatman paper in a stainless steel holder, and washed twice with 2 cc portions of 2% TCA containing NaH_2PO_4 (3 mg P/ml). The precipitates and paper were digested with 15 ml concentrated HNO_3 to small volume, then treated with 2 ml of concentrated HCl and water. After making up to volume the TCA filtrates and insoluble fractions were counted using a dipping counter.

A third group of 16 animals was employed in order to determine the effect of increasing the weight of the uterus on the uptake of P^{32}. The increased uterine weight in this group was accomplished by injecting the estradiol in oil in divided doses (Table III). These animals were given a total of 0.5 γ of estradiol in oil, divided into 3 daily injections. On the first

TABLE II. Fractionation of Uteri into Acid-Soluble and Acid-Insoluble Portions.

Total No. animals	Run No.	Avg time in hr	Avg uterine wt in g	Acid-soluble		Acid-insoluble		Inj.
				% dose × 10^3	% dose/g	% dose × 10^3	% dose/g	
2	1	3	.04	18	.47	10	.26	P^{32}
2	1	3	.06	35	.59	17	.28	" + Est.
2	1	6	.03	18*	.58*	6	.20	"
2	2	6	.04	17	.43	8	.21	"
2	1	6	.06	9	.16	23	.38	" + Est.
2	2	6	.06	51	.86	16	.27*	" + Est.
2	1	24	.04	6	.17	22	.59	"
2	2	24	.04	17	.46	14	.36	"
2	1	24	.06	21	.32	56	.86	" + Est.
2	2	24	.08	38	.45	79	.93	" + Est.
2	1	48	.04	9	.21	13	.33	"
2	2	48	.03	9	.26	10	.30	"
2	1	48	.07	21	.31	60	.89	" + Est.
2	2	48	.05	22	.43	44	.87	" + Est.
2	1	72	.03	8	.24	12	.35	"
2	2	72	.04	8	.18	15	.33	"
2	1	72	.05	9	.32	43	.91	" + Est.
2	2	72	.04	15	.38	28	.69	" + Est.

* Some loss.

TABLE III. Effect of P^{32} Uptake by Increasing Uterine Weight Through Priming with Estradiol in Oil.*

Total No. animals	Avg uterine wt in g	Standard error	% dose × 10^3	% dose/g	Staud. error	Injection†
6	.029	±.002	23	0.79	±.07	P^{32}
10	.105	±.003	139	1.33	±.06	P^{32} + Est.

* Animals killed 72 hr after inj. with P^{32}.
† Total dose employed = P^{32} 5 μc.
Estradiol 0.5 γ.

day of the injection of the estradiol, 5 μc of P^{32} was injected separately in an aqueous solution. They were then injected for 2 more days with estradiol in oil and were killed on the fourth day with chloroform.

The livers were removed in one group of animals (Table IV) with a view toward determining the uptake of P^{32} by the liver in estradiol treated animals. The same methods were employed in this group as in the previously mentioned groups of rats.

Discussion. The estrogenic hormones are known to have a stimulating effect on vaginal mucosa, endometrial epithelium as well as on the acinal epithelium of the breast. The proliferation thus produced would tend to increase the uptake of phosphorus made available to the animal organism. This idea received some support from the preliminary report of Hertz(4) who observed an augmented uptake of P^{32} in cancer tissue of the breast in humans who had been treated previously with testosterone because of breast malignancy.

The data in Table I indicate that early in the experiment the average uptake of P^{32} was greater in the uteri of the rats treated with estradiol plus P^{32} than in the rats that received P^{32} alone. The uptake of P^{32} by the estradiol plus P^{32} treated rats increased with time, reaching the highest concentration at the end of 48 hours. On the other hand, the uteri of the animals that were injected with P^{32} alone showed significantly lower activity. The concentration of P^{32} in these uteri remained fairly constant for the duration of the experimental time.

The data recorded in Table II indicate that there is increased binding of the administered P^{32} within the target organs of animals treated with estradiol. The acid insoluble fraction in the estradiol treated rats shows low activity in the first few hours. There is a rapid increase in the first 24 to 48 hours that is well maintained through 72 hours. When P^{32} is injected alone the per cent dose per gram of

4. Hertz, Saul, and Rooney J. Stewart, *Abst. Fed. Proc.*, 1950, v9, 334.

TABLE IV. Uptake of P^{32} by the Liver in Estradiol Treated Animals.

Time in hr	% dose/g	Injection
6	2.23	P^{32}
6	1.85	" + Est.
24	1.57	"
24	0.98	" + Est.
48	1.19	"
48	1.76	" + Est.
48	2.47	"
48	2.28	" + Est.

tissue in the acid insoluble fraction is lower in the early hours than when P^{32} and estradiol are injected together. The acid soluble fraction, when P^{32} is injected alone, shows higher activity in the first few hours than does the acid insoluble fraction. It then drops to a fairly constant level which is maintained through 72 hours. No evident explanation for this is apparent.

In order to determine whether we could increase the uptake of P^{32} by further increasing the weight of the uterus an experiment was designed toward that end. Estradiol in oil was injected for three successive days, in place of a single injection of aqueous estrogen, so that an average uterine weight of 104 mg was accomplished (Table III). In spite of a 4- to 5-fold weight increment in the uterus of the animals thus treated the uptake of P^{32} by the organ was no greater than in those animals (Tables I and II) with smaller uteri in consequence of a single injection of aqueous estradiol.

Since Zondek(5) has shown that estradiol is inactivated in the liver the question arose whether the combined use of P^{32} and estradiol may be reflected in that organ. Reference to Table IV indicates that there is no preferential localization of P^{32} in the liver. The process of the intermediary metabolism of estradiol apparently plays no part in the localization of the P^{32}. When, however, the estradiol is instrumental in stimulating cells to proliferation, as in a target organ such as the uterus and the breast, the phosphorus becomes incorporated within the cells in greater concentrations than under normal conditions.

The data in this report suggest the important possibility that therapeutic agents having a short half life might be expected to exercise an inhibitory effect on certain neoplastic tissue if the latter is a part of a target organ, the growth of which can be stimulated by a specific agent. P^{32} emits the beta ray which has the capacity to produce ionization in tissues and has a maximum range of penetration of approximately 7 mm(3). We are currently employing the combined use of a specific steroid with P^{32} as a means of therapy in malignancy of the genital tract. The evaluation of the application of this principle awaits further study.

Summary. 1. The preferential localization of radioactive phosphorus is enhanced in the uterus of the immature rat by the administration of estradiol. 2. P^{32} becomes bound in the estradiol primed uterus as evidenced by the increase of P^{32} in the acid insoluble fraction. 3. The liver is not influenced in this process, indicating that the proliferating cells in a hormone treated target organ influence the uptake of P^{32}. 4. The data indicate a suggested approach to the treatment of genital malignancies.

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5. Zondek, B., *Hormone des Ovariums und des Hypophysenvorderlappens,* 2nd Ed. Springer, Vienna, 1935, 124.

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