Distribution and Excretion of Radioactivity After Parenteral Administration of Radioactive Polydiethylstilbestrol Phosphate to Rats and a Cow. (29590)

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Polydiethylstilbestrol phosphate (PSP) is a high molecular weight polyester of phosphoric acid and diethylstilbestrol(1). The compound is water soluble and its molecular weight is about 14,000. It has been shown to produce a prolonged estrogenic effect when injected parenterally in spayed mice. Orally, its estrogenic activity was negligible. PSP is a very potent inhibitor of certain enzymes, such as acid and alkaline phosphatase, hyaluronidase and β-glucuronidase. Extensive field trials have shown that PSP is an effective growth-promoting agent for cattle(2). A single injection of 250 mg of the polymer administered to steers 3 to 4 months before slaughter significantly improved weight gains and feed efficiency. In mice the distribution of PSP labeled with tritium and C^{14} has been studied by autoradiography(3). Radioactivity was preferably accumulated in tissues containing reticuloendothelial cells such as the liver, spleen, bone marrow and lung.

The purpose of the present study was to investigate the tissue localization and rate of excretion of radioactive material after parenteral administration of radioactive PSP to rats and a cow.

Materials and methods. According to Wilzbach(4) 200 mg of PSP was exposed to 1 C of tritium gas at room temperature for 14 days. The tritiated compound (H^3-PSP) was purified as follows. The substance was dissolved in 0.1 M NaOH and boiled for 2 hours. The solution was then dialyzed against running water for 48 hours. H^3-PSP was precipitated by dilute HCl and dried over P_2O_5 *in vacuo* to constant weight. The randomly labeled H^3-PSP had a specific activity of 40 μc/mg, which was not changed after repeated boiling and dialyzing.

The biological activity of the tritium-labeled compound was found to be the same as that of non-labeled PSP. As an index of response, the prolonged duration of estrogenic effect was estimated by the mouse vaginal smear technique(5).

The antienzymic property of H^3-PSP was identical with that of the non-labeled compound, when the 2 preparations were tested as to their inhibitory action on testis hyaluronidase according to a method described earlier(6).

C^{14}-labeled PSP (C^{14}-PSP) with a specific activity of 10 μc/mg was later prepared from diethyl(ethyl-1-C^{14})stilbestrol.*

Determination of radioactivity in tissues, blood and excreta. The samples to be analyzed were prepared for counting in the following way. Aliquots of 5-10 g of different tissues and feces were disintegrated in 50 ml 0.2 M NaOH by heating to boiling and stirring for 2 hours. After dilution to 100 ml with water, the hydrolysates were extracted with 100 ml portions of n-butanol until no more radioactivity was recovered.

The radioactive material in urine and blood was extracted directly with butanol. Radioactivity was measured in planchets using a Frieseke-Hoepfner windowless gas-flow counter. Two aliquots of each extract to be analyzed were plated. The counting efficiency, when measuring the radioactivity in different extracts, varied usually from 16 to 30% depending mainly on the dilution of the extracts.

To evaluate the efficiency of the extraction technique, recovery experiments were carried out. It was found that between 40 and 60% of the radioactivity added to the different materials was recovered by the methods used.

Unless otherwise stated, all values reported are corrected for background radiation, self-absorption and for the losses due to the incompleteness of the extraction technique.

Results. Intravenous injection of H^3-PSP in rats. Four rats were injected (tail vein)

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with 90 μg of H^3-PSP corresponding to 7.0 $\times$ 10^6 cpm. The animals were sacrificed 24 hours later and the livers removed for separate analysis of radioactivity. It was found that 3.6 $\times$ 10^6 (3.4-3.8) cpm (approx. 50%) was accumulated in the liver.

The radioactivity present in the liver exhibited a very slow disappearance rate. About 15% of the administered radioactivity was recovered from the liver 28 days after an intravenous injection.

Intramuscular injection of H^3-PSP to rats. Forty-five rats were injected intramuscularly (right hind leg) with 45 μg H^3-PSP (3.4 $\times$ 10^6 cpm). On days 1, 5, 11, 18, 25, 39, 53, 64 and 90 after injection, groups of 5 animals each were sacrificed. Since 2 rats died, the last 2 groups contained only 4 animals each. The livers and the right hind legs were extirpated and the blood collected.

At varying intervals, urine was collected for 24 hours from 2 rats in each group and analyzed for radioactivity.

The results obtained are summarized in Fig. 1-4.

The data of Fig. 1 indicate that the bulk of radioactivity was accumulated at site of injection. About 70% was recovered from the injected leg 24 hours after the intramuscular injection. The rate of disappearance was slow since as late as 90 days after the injection about 20% of the administered radioactivity remained at site of injection.

Radioactive material was rapidly accumulated in the liver after an intramuscular injection of H^3-PSP (Fig. 2). At 5 days the highest accumulation was found, which was about 6% of the injected dose. The radioactivity disappeared slowly; 64 days after the injection there still were detectable amounts of radioactivity in the liver.

The excretion pattern of urine (Fig. 3) was characterized by a high level of radioactivity delivered before the fourth day after the injection. After 42 days the radioactivity was too low to be estimated.

The initial high concentration of activity in blood (Fig. 4) declined slowly and after 39 days only trace amounts were detectable. The radioactivity data presented in Fig. 3 and 4 are not corrected for losses due to the extraction technique and self-absorption. Therefore, these curves only indicate the relative amounts of radioactivity at different times of the investigation period.

Experiments with C^{14}-PSP in rats. Part of the investigation carried out with H^3-PSP on rats was repeated when C^{14}-labeled PSP was available. The results obtained were in close agreement with those reported above, and will therefore not be described in detail.

Subcutaneous injection of H^3-PSP to a cow. An 1100 lb Holstein cow was injected subcutaneously at the base of the ear with 212 mg of H^3-PSP (11 $\times$ 10^9 cpm) dissolved in 5 ml of water. Urine and blood were collected daily for 15 days and 3 fecal samples were also obtained.

After slaughter (15 days after injection), liver, spleen, kidney, brain, pituitary gland, lung, thyroid, ovary and muscle were analyzed.

Urine. The excretion of radioactivity in the urine at different intervals after injection is shown in Fig. 5. The first sample of urine, collected one hour after the injection, was found to contain a high amount of radioactivity. Assuming the daily excretion of urine to be 15 liters, the total amount of radioactivity excreted in the urine during the 15 experimental days was calculated to be 7.5 $\times$ 10^8 cpm, approximately 7% of the administered dose.

Blood. Samples of 250 ml blood were drawn daily from the cow and immediately oxalated and extracted as described. The disappearance of radioactivity from blood during the experiment is shown in Fig. 6. The highest level of radioactivity was found 3 days after the injection. Calculating the blood volume to be 33 liters(7), the total radioactivity present in the blood on the 15th day of the experiment was 3.3 $\times$ 10^8 cpm, approximately 3% of the injected dose.

Feces. Three fecal samples collected on days 3, 8 and 15 were analyzed for radioactivity. Extracts of these samples contained high amounts of radioactive material. Estimating the daily output of feces to be 12 kg it was calculated that approximately 14%

of the administered radioactivity was excreted by this route.

Different organs. Among the organs analyzed (Table I), liver, spleen, kidney and lung were found to contain appreciable amounts of radioactivity. Samples from other organs contained no labeled material (muscle, brain, thyroid) or only traces (pituitary

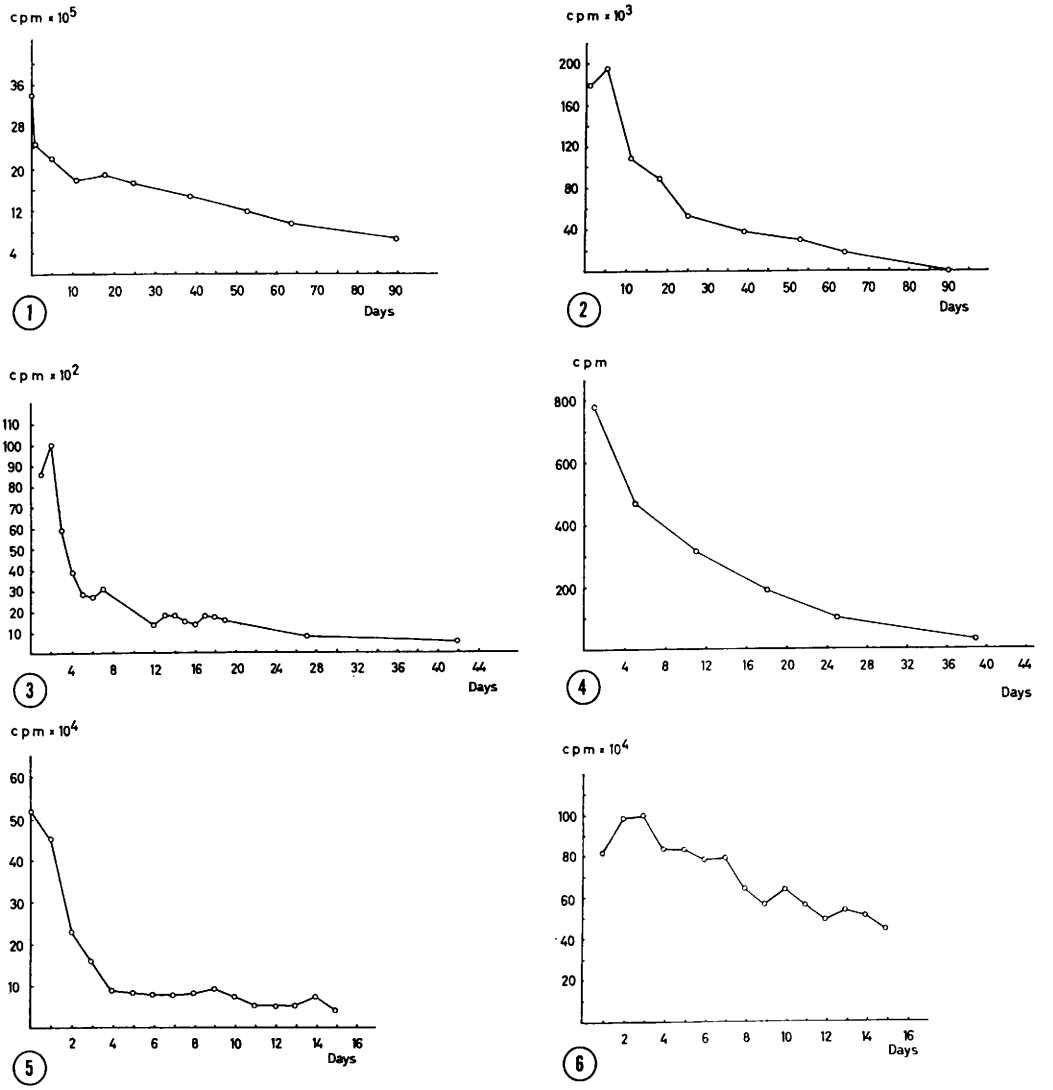

FIG. 1. Disappearance of radioactivity from injection site of H^3-PSP-treated rats after intramuscular injection (3.4×10^6 cpm). Each point represents mean value for 5 animals.

FIG. 2. Disappearance of radioactivity from liver of H^3-PSP-treated rats after intramuscular injection (3.4×10^6 cpm). Each point represents mean value for 5 animals.

FIG. 3. Excretion of radioactivity in the urine of H^3-PSP-treated rats after intramuscular injection. Curve indicates only relative amounts of radioactivity in urine at different intervals after injection.

FIG. 4. Disappearance of radioactivity from blood of H^3-PSP-treated rats after intramuscular injection. Curve indicates only relative amounts of radioactivity in blood at different intervals after injection.

FIG. 5. Radioactivity in extracts of 50 ml of urine from a cow injected subcutaneously with H^3-PSP (11×10^9 cpm).

FIG. 6. Radioactivity in extracts of 50 ml of blood obtained from a cow injected subcutaneously with H^3-PSP (11×10^9 cpm).

TABLE I. Recovery of Radioactivity from Excreta, Blood and Organs of a Cow After a Subcutaneous Injection of 11×10^9 cpm of Tritium-Labeled Polydiethylstilbestrol Phosphate.

Material	Recovered radioactivity		
	cpm	cpm/g tissue	% of inj activity
Blood*	—	—	3 §
Urine†	—	—	7 §
Feces‡	—	—	14 §
Liver	140×10^7	30×10^4	12.7
Spleen	6×10^7	7×10^4	.5
Kidney	17×10^7	15×10^4	1.5
Lung	9×10^7	8×10^4	.8
Site of inj	560×10^7	—	50.9
Thyroid	0	—	
Pituitary gland	27×10^3	—	
Ovary	11×10^4	—	
Muscle	0	—	
Brain	0	—	

* Blood volume is assumed to be 33 l.
† Daily excretion of urine is assumed to be 15 l.
‡ Daily excretion of feces is assumed to be 12 kg.
§ Approximate values.

gland, ovary). The injection site (ear) had approximately 50% of the injected radioactivity.

All results are summarized in Table I. About 90% of the injected radioactivity was accounted for.

Discussion. The most interesting findings obtained in the investigation on rats seem to be (1) the very high accumulation of radioactivity in the liver after an intravenous injection, indicating the significant role played by this organ in removing PSP from circulating blood, (2) the depot at site of injection, and (3) the very slow disappearance of radioactivity from the body of the treated rats. It seems reasonable to associate these findings with the greatly prolonged estrogenic effect of PSP, earlier reported. The choice of a cow as the experimental animal in the second part of this investigation was suggested by the encouraging results obtained with PSP as a growth-stimulator for cattle. It was felt that an investigation on the distribution and excretion of radioactivity after an injection similar to that carried out in the field might throw some light on the mechanism by which the drug exerts its prolonged action.

The results obtained were in good agreement with the results of the rat experiments. The initially high concentration of activity in urine and blood, the depot at site of injection, and the accumulation of radioactivity in the liver seem to be similar in both species.

The more detailed distribution pattern of radioactivity in the cow corresponded very closely to that obtained by Bengtsson *et al* (3) in mice, *i.e.* the radioactivity was particularly accumulated in tissues containing reticuloendothelial cells.

The finding of a depot at site of injection, containing half of the injected dose 15 days after injection, suggests that the polymer is primarily accumulated at this place and that the different RE depots are continuously supplied with PSP from here. However, the fact that PSP, administered intravenously to rats, was accumulated in the liver and disappeared very slowly from this organ, indicates that the RE depots may contribute in the prolonged estrogenic effect exerted by PSP.

The finding of a very slow disappearance of radioactivity from the body of the treated animals explains the long duration of effectiveness reported for the drug in cattle. This slow disappearance may, however, constitute a problem as to the use of the compound as a growth-stimulating agent, since it seems likely that minute amounts of PSP may be retained in edible parts of the treated animals.

This brings up the question of the absorbability in the digestive tract of the high molecular weight compound. Investigations on this will soon be reported.

Summary. Polydiethylstilbestrol phosphate (PSP), a water soluble polyester of phosphoric acid and diethylstilbestrol, was injected in rats and a cow and the distribution and excretion of radioactivity were followed in both species. After a subcutaneous or intramuscular injection the bulk of the radioactivity was accumulated at the site of injection. The disappearance of radioactivity from this depot was very slow. As late as 90 days after the injection into rats, 20% of the administered radioactivity was still accumulated at the site of injection. The liver was very effective in removing labeled material from circulating blood. Twenty-four hours after intravenous injection in rats, about

50% of the radioactivity was recovered from this organ. The distribution of radioactivity in the cow indicated that the polymer was preferably accumulated in organs containing reticuloendothelial cells.

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Effect of Nonionic Detergents on Electrophoretic Distribution of I-131-Labeled-Thyroid Hormones in Serum.* (29591)

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During the course of studies concerned with the effects of nonionic surface-active agents on the electrophoretic properties of serum proteins, striking changes were noted in the migration patterns of I-131-labeled thyroid hormones. Consequently, a series of experiments was carried out in which varying concentrations of nonionic detergents were examined for their abilities to alter the electrophoretic partition of thyroxine-I-131 and triiodothyronine-I-131 among serum proteins.

Materials and methods. Either Triton X-100† (a nonionic alkylphenoxy polyethoxy ethanol) or Tween 80 (polysorbate 80) was added in varying concentrations to 0.5 ml aliquots of normal serum. These were followed by addition of 0.01 ml of a 1% solution of human serum albumin containing 1.0 μc of I-131-labeled-l-thyroxine or -l-triiodothyronine‡ after which the entire mixture was stirred vigorously with a wooden rod. Triton X-100 was used without further dilution, but because of its viscosity, it was necessary to dilute the Tween 80, 1:3 with distilled water.

Zone electrophoresis was carried out on strips of Whatman No. 3 filter paper (1¼ × 18½ inches) folded in half and suspended vertically over a glass rod in a closed lucite cell. The free ends of the paper extended 1¼ inches into baffled compartments containing platinum wire electrodes and barbital buffer (pH 8.6, ionic strength 0.075).

Ten to 20 microliters of the serum mixture were applied in a narrow band along the apex of the paper and covered with the buffer solution (the paper had been previously saturated with barbital buffer except for the region at the apex). An electric potential of 90 to 110 volts (6 to 9 ma) was applied for approximately 18 hours at room temperature, after which the paper strips were removed, dried in air and the radioactivity on the paper was located by means of a strip scanner connected to a continuous recorder or by autoradiography using X-ray film.

The proteins on the paper were stained

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† Rohm and Haas, Co., Phila., Pa.

‡ Abbott Laboratories, Oak Ridge, specific activity, 25-40 mc/mg.