The presence of progesterone in the sow has been established for corpora lutea (Loy et al., 1958; Erb et al., 1962), adrenals (Erb et al., 1962), peripheral blood plasma during pregnancy (Short, 1960) and ovarian vein blood plasma during the estrous cycle (Gomes et al., 1965). Marker and Rohrmann (1939) have reported the presence of pregnanolones in extracts of sow urine, but did not find pregnanediols. Mayer et al. (1961) reported quantitative estimates of pregnanediol and a conjugated metabolite, but did not report proof of identity of the compounds.

The excretion of progesterone metabolites has been demonstrated to vary widely among species depending upon the mode of inactivation and conjugation. Experiments utilizing radioactive progestins have shown that radioactivity appears predominantly in the feces of the cat (Taylor, 1964), cow (Gassner et al., 1960; Williams, 1962), mouse and rat (Riegel et al., 1950) and sheep (Ogilvie et al., 1962). The chimpanzee (Romanoff et al., 1963a, b), human (Davis and Plotz, 1958; Sandberg and Slauengwhite, 1958: Romanoff et al., 1963c) and rabbit (Cooke et al., 1963) excrete significant amounts of radioactivity in the urine. In a brief report Schomberg et al. (1964) have shown that the sow also excretes metabolites of progesterone in the urine.

This study was undertaken to estimate the rate and quantity of intravenously administered progesterone-4-14C excreted by the porcine kidney.

Materials and Methods

Nine microcuries representing 166 mcg. of commercially prepared, chromatographically pure progesterone-4-14C were intravenously administered via the vena cava to a sow on day 13 of one cycle and day 19 of the next cycle.

Preparation of Radioactive Material. Since the steroid obtained from the manufacturer was dissolved in benzene, the preparation was dried and redissolved in ethanol to yield a concentration of 9 mcg. per ml. of ethanol. One milliliter plus 1.5 ml. of "rinse" ethanol were transferred to an injection vial with a tuberculin syringe. Immediately prior to injection, sterile saline containing 10%, ethanol was used to reduce the concentration of the ethanol containing the steroid. This was done with a repeating syringe apparatus which allowed saline-10% ethanol from a separate source to be drawn into the syringe containing the dissolved steroid plus an additional 3 ml. of ethanol wash, which was used to rinse the injection vial. Therefore, when the repeating syringe was attached to the saline source, a closed system was achieved which allowed continuous infusion of the radioactive preparation and two saline-10% ethanol rinses without disassembling the injection apparatus. Injection was completed within 5 to 5.5 min., and the total fluid volume injected was 15 to 16 ml. The injection vial and syringe were later rinsed with ethanol and checked for residual radioactivity. This amount was subtracted from the calculated injected dose.

Animal Preparation and Collection. In order to minimize stress to the animal and possible confounding effects of the adrenal steroids, the sow was prepared 4 hr. prior to injection. A PE-90 polyethylene cannula was inserted into the anterior vena cava, filled with heparin and taped to the neck of the animal for easy manipulation. Also, a size 12 Carson Model Foley retention catheter was inserted into the bladder. A pre-injection control sample of urine was collected over a 4-hr. period.

At the time of injection the sow was unrestrained and lying down; she remained so throughout the next 4 hr. in both injection trials.

During and after injection, urine collections were made at 2-min. intervals for the first 40 min., at 10-min. intervals until 60 min., at 30-min. intervals until 2 hr., and hourly there-
after until 12 hr. postinjection. Collections were then made at 6-hr. intervals until the catheter passed at 2 days for injection 1 and clogged at 6 days for injection 2; thereafter, all urine was collected as voided (using Gooch tubing cemented around the vulva) until background levels were reached.

In the first trial a tranquilizer (promazine) was administered at the time of catheterization; in the second, none was used. To minimize infection, the bladder was infused daily via the catheter with 0.2 gm. penicillin and 0.375 gm. streptomycin sulfate (crystalline) dissolved in 6 ml. saline; the catheter tubing was clamped off for 20 to 30 min., and free urine flow was then allowed to resume.

Incomplete Collections. When urine loss occurred during the postcatheter period, volume corrections were made on a creatinine excretion basis using the method of Owen et al. (1954).

Counting Procedure. A xylene-dioxane-cellosolve (XDC) liquid scintillation medium which allowed direct counting of urine aliquots was used. The scintillation liquid was composed of 1 part xylene, 3 parts dioxane, 3 parts cellosolve (2-ethoxyethanol), 1.0% PPO (2,5-diphenyloxazole), 0.05% POPOP [1,4-bis-(5-phenyloxazolyl)] and 8.0% naphthalene. The procedure as outlined by Bruno and Christian (1961) was modified by using only 1 ml. of urine per 15 ml. XDC and deleting the hydrogen peroxide decolorization step. The degree of quenching was calculated individually for each sample by adding a known amount of progesterone-4-14C in ethanol as internal standard to a second counting vial. The ethanol-containing internal standard was dried before urine was added. After correction for quenching, multiplication of the observed radioactivity per milliliter of urine by the total volume of urine collected gave the total radioactivity for each period. Samples were counted three times for 10 min. each in a Packard Tri-Carb liquid scintillation counter at a high voltage tap of 4.0, discriminator settings of 100 to 1000 for both channels and gain controls of 10–10–10.

Results and Discussion

Excretion of Radioactive Metabolites. Data for the two injections were averaged to prepare figures 1 and 2. The curve in figure 1 represents an average of radioactivity in urine at each collection period as described earlier.

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The data in figures 1 and 2 and table 1 show that the porcine kidney is an important route of progesterone excretion. Metabolism and elimination were rapid, since radioactivity was found in the urine 6 and 8 min., respectively, after initiation of injection in the two trials. Radioactivity was near maximum 20 min. after injection, then declined before reaching a second peak at 40 min. (figure 1). The decline in radioactivity between 20 and 40 min. was similar for each injection and was lowest between 26 and 30 min. for each injection (table 1.). At the end of the first day 28% of the administered dose had been excreted in the urine.

Over 50% of the total activity excreted in the urine was eliminated during the first 24 hr. (figure 2). The cumulative percentages of excreted radioactivity are listed separately for injections 1 and 2 in table 1, because the catheter was passed during the second day for injection 1 and urine was collected totally as voided thereafter. Radioactivity elimination
was virtually identical for both injections. On this basis the rate of progesterone metabolism appears to be similar for mid- and late stages of the estrous cycle.

A total of 48.5% of the injected dose could be accounted for as urinary radioactivity for injection 2 (table 1). Although elimination was most rapid during the first 24 hr., the compound was presumably in equilibrium with plasma progesterone and slowly diffusing from the body since urine counts did not reach background until day 10 for injection 2.

The excretion pattern observed in this study coincides with similarly designed studies in the human in that most of the radioactivity was excreted within 4 days following administration of the hormone, but some radioactivity was still detectable until days 10 to 13 post-injection (Davis and Plotz, 1958; Harkness and Fotherby, 1963).

General Discussion

The results obtained with the sow concur with the concepts which have emerged from tracer studies in the human, namely: inactivation by the liver and kidney, diffusion of steroid into body tissues, and excretion of radioactivity in the urine (Zander, 1959, 1961; Pearlman, 1960; Davis and Plotz, 1958). Zander (1959) reported an accumulation of 170% of administered radioprogesterone in urine within 30 min. following intravenous injection. After only 9 min. most of the radioactivity was contained in the conjugated plasma steroids, and hydrolysis of this fraction yielded predominantly pregnanediol and some 5β-pregnane-3α,20-one. In a similarly designed study Davis and Plotz (1958) reported that 11% of the radioactivity from progesterone-21-14C was eliminated via the kidneys, liver and lungs within 3 hr., whereas only 5% of the dose remained in the plasma; they concluded that over 80% of the radioactivity diffused into the tissues and fat stores. In the ewe Short and Rowell (1962) found a similar but more dynamic situation; within 5 min. following injection, almost 99% of the dose had been removed from the blood.

Summary

Nine microcuries representing 166 mcg. of progesterone-4-14C were intravenously administered to a sow on day 13 of one estrous cycle and day 19 of another estrous cycle to determine if urine is a significant route of progesterin elimination by the porcine. Radioactivity was present in the urine within 6 min. following injection. Over 50% of the total activity excreted in the urine was eliminated within the first 24 hr., but background levels were not observed until 10 days after injection. Total urinary radioactivity accounted for 48.5% of the administered dose. Differences in excretion pattern due to stage of the estrous cycle were not apparent.

Literature Cited


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**TABLE 1. HOURLY CUMULATIVE EXCRETION OF RADIOACTIVITY IN URINE FOLLOWING ADMINISTRATION OF NINE MICROCURIES OF PROGESTERONE-4-14C**

<table>
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<th>Excreted (%)</th>
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<th>Excreted (%)</th>
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<td></td>
<td>240</td>
<td>48.5b</td>
</tr>
</tbody>
</table>

*Total collection terminated.

b Background level.
Taylor, W. 1964. 4-14-C-progesterone metabolism in the rabbit. Biochem. J. 90:30P.