to be inhibited by AOAA *in vitro*(13-16) and in certain cases it appeared that the inhibition was mediated through the aldehydic form of vit. B_6(15,17) findings which are in good agreement with our original postulation that AOAA toxicity was related to its chemical interaction with pyridoxal. Our present studies indicate also that in the case of AOAA-induced vit. B_6 deficiency, all 3 urinary metabolites studied may serve as valid indices for a vit. B_6 deficiency.

Summary. Chronic administration of aminooxyacetic acid (AOAA) to rats after a tryptophan load resulted in an increase in xanthurenic acid and oxalic acid excretion and a decrease in pyridoxic acid excretion, thus suggesting a vitamin B_6 deficiency. Subconvulsive doses of the drug resulted in a partial depletion of liver, but not brain, pyridoxal phosphate and pyridoxamine phosphate. Thus, it would appear that increased xanthurenic acid excretion ofter a tryptophan load is a valid index for measuring a vitamin B_6 deficiency induced by chronic administration of aminooxyacetic acid.

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Progesterone Biosynthesis in Perfused Corpora Lutea.* (31483)

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Studies on metabolism of the ovary indicated that it would be useful to have an *in vitro* system which resembled the *in vivo* corpus luteum with regard to retained anatomical structure, blood supply and compartmentalization. The *in vitro* system was accomplished by isolating the luteal phase ovary and perfusing it through the ovarian artery with a blood substitute(1). The blood substitute was used to reduce or remove the organ from luteotropins and other substances present in the blood which regulate ovarian metabolism. Organs perfused in this manner were found to utilize oxygen for several hours and incorporate acetate-1-C^{14} into ether soluble material(1). This report offers evidence that the labeled acetate in the perfusate was also incorporated into progesterone.

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Materials and methods. Progesterone and other steroids were purchased from Sigma Chemical Co., St. Louis, Mo. Progesterone-7 α-H^3 (1,300 mc/mmole) was obtained from Nuclear-Chicago Corp., Des Plaines, Ill. The tritium labeled progesterone was stored as supplied in methanol solution at —20°. Decomposition of the tritium labeled compound on storage under these conditions does not exceed 5% per annum. Sodium acetate-1-C^{14} (41.3 mc/mmole) was purchased from New England Nuclear Corp., Boston, Mass. Silica Gel G-HR/UV (Machery, Nagel & Co.) was purchased from Brinkman Instruments, Inc. Perfusion pressure and oxyhemoglobin concentrations were monitored by means of a Bourdon type pressure transducer and physiograph MK III flow-through oximeter sold by E and M Instruments Co. of Houston, Texas.

Gas chromatography was done with a Barber-Coleman model Selecta-5000 instrument equipped with a model A-5042 argon ionization detector. Solid support (Gas chrom Q) and the silicone gum stationary phases (DC QF-1, GE XE-60 and GE SE-30) were purchased from Applied Science Laboratories, College Station, Pa. Chromatographic technique using the solid support coated with 1% SE-30, 1% XE-60, or 3% QF-1 was similar to procedures previously reported(5, 6,7).

Liquid scintillation spectrometry was accomplished with a Packard Tricarb model 314-B (Packard Instruments, La Grange, Ill.). Carbon-14 activity was separated from tritium activity by counting at two high voltage settings. Carbon-14 was counted at high voltage 900 and discriminator setting 200-1000 resulting in a counting efficiency of 0.48 for carbon-14 and 0.0013 for tritium. Tritium was counted at a high voltage setting of 1120 and discriminator settings of 100-200 resulting in a counting efficiency of 0.14 for tritium and 0.05 for carbon-14. Samples containing both tritium and carbon-14 required no correction for determination of carbon-14 activity. Determination of tritium activity in samples containing both carbon-14 and tritium required an initial determination of carbon-14 activity and a correction for carbon-14 contributions to the total activity.

Freshly collected luteal-phase swine ovaries were flushed at 12° with a sodium citrate, citric acid, sodium chloride and dextrose solution adjusted to pH 7.4(2). The ovaries were held at 12° until placed in the perfusion chamber at 37° and perfused through the ovarian artery. The 300 ml of perfusate used to perfuse each ovary was cell culture medium(3) containing washed erythrocytes from castrated male swine (hematocrit 40%), 200 IU human chorionic gonadotropin, acetate-1-C^{14} (3.7×10^{-4}M, 0.17 μc/μmole), 1.5×10^5 units penicillin, 180 mg streptomycin, 3 g egg albumin, 6 g dextran and 300 mg pilocarpine hydrochloride. Perfusions were carried out in a perfusion apparatus described by Vandemark and Ewing(4) modified to permit oxygenation of larger volumes of perfusates, monitoring of perfusion pressure and monitoring of arterial-venous oxyhemoglobin concentrations.

The ovaries were perfused from one to three hours and the corpora lutea removed, weighed and homogenized in 0.9% NaCl to a final volume of 30 ml. Red blood cells were removed from the perfusate by centrifugation and both the perfusate and homogenate of the corpora lutea were stored at —20° until the progesterone was isolated by a modification of published procedures(8,9).

The perfusate and homogenate of corpora lutea were extracted either with diethyl ether or hot acetone after 13.2×10^{-3} μc of 7 α — H^3-progesterone was added to estimate recoveries. Ether extraction of progesterone was more cumbersome, but it allowed an immediate removal of estrogens from the lipid material. The removal of estrogens was a desirable feature for other experiments. For the isolation of progesterone in these experiments, hot acetone extraction was simpler and the preferred procedure, but both extraction procedures were satisfactory and gave comparable results.

For ether extraction 100 ml of the perfusate or 27 ml of the luteal tissue homogenate was diluted to 250 ml with 0.9% NaCl. The pH was adjusted to 10 with N NaOH and the material extracted 4 times using 300 ml of ether for each extraction. The combined

TABLE I. Progesterone Quantities in Perfusate and Corpora Lutea from Perfused Ovaries.

Exp	Total progesterone†	Progesterone in corpora lutea*	% Total	Progesterone in perfusate*	% Total	Net synthesis of progesterone‡	% Total
1	107	101	94	6	6	41	38
2	66	58	86	9	14	40	61
3	103	97	94	6	6	28	27
4	97	85	88	12	12	23	23
5	37	37	100	<1	3	0	0
6	26	26	100	<1	3	0	0
7	50	41	82	9	18	0	0
8	225	192	85	33	15	174	78
9	46	42	91	4	9	0	0
10	70	67	96	3	4	60	86
11	82	82	100	<1	<1	36	44
12	94	84	89	10	11	28	30
13‖	49	43	88	6	12	42	86
14‖	18	12	77	6	33	5	28
15‖	70	65	93	5	7	19	27
16‖	112	112	100	<1	<1	18	16
Average	78	72	92	6	8	43§	45§

* Progesterone quantities in the perfusate and corpora lutea are expressed as progesterone per g luteal tissue.

† Total progesterone = perfusate progesterone and corpora lutea progesterone.

‡ Net synthesis of progesterone = total progesterone minus progesterone content of the non-perfused ovary, taken from the donor animal. Progesterone concentration in both ovaries taken from the same animal have been found to be the same before perfusion(13).

§ Value for corpora lutea showing a net synthesis of progesterone.

‖ The perfusate contained no human chorionic gonadotropin.

ether extract was washed twice with 30 ml water and evaporated to dryness under reduced pressure. An alternative procedure was to add 15 volumes of acetone at 50° to the perfusate or luteal tissue homogenate and filter. Acetone was removed from the filtrate by evaporation under reduced pressure and the aqueous residue was extracted 3 times with 10 ml ether. The ether extract was taken to dryness under reduced pressure.

Residues obtained from either extraction procedure were then dissolved in 2 ml of a solution of methanol-water (7:3) and the mixture was extracted several times with 2 ml portions of ligroin. Methanol was removed by evaporation under reduced pressure and the volume of the aqueous residue adjusted to 0.5 ml with water. The aqueous residue was extracted 3 times with 1.5 ml diethyl ether. Progesterone was isolated from the ether extract by thin layer chromatography using chloroform—methanol (99:1) as the solvent. Some samples required a second purification by thin layer chromatography using the same solvent or chloroform—methanol (95:5). For 48 samples progesterone recoveries were 35% ± 22% using this procedure.

Results and Discussion. Progesterone obtained in this manner chromatographed as a single spot in the thin layer systems. The 2,4-dinitrophenylhydrazones of the progesterone were prepared and identified by thin layer chromatography(10,11). An ultraviolet absorption spectrum of the isolated progesterone showed the characteristic absorption maxima at 240 mμ and the spectrum was identical to the spectrum of a progesterone standard. Gas-liquid chromatography with 1% SE-30, 1% XE-60 or 3% QF-1 was carried out on the isolated progesterone. The stationary phases chosen show quite different retention effects for steroids(6). For example, cholesterol follows progesterone in its appearance from the column when SE-30 is used as the stationary phase but the reverse situation is found when QF-1 is used. Chromatography of the isolated progesterone using the stationary phases produced only a single major peak having the same retention time of a standard progesterone sample.

As may be seen in Table I, progesterone was present both in the perfusate and corpora lutea. Since all progesterone values are expressed as quantities per gram of luteal tissue,

it may be seen that some ovaries were as much as 12 times more active than others in production of progesterone per gram of luteal tissue. Of the total progesterone 88% ± 12% was found in the corpora lutea with the remainder in the perfusate. Corpora lutea, showing a net synthesis of progesterone, synthesized 43 μg of progesterone per gram of luteal tissue during the perfusion period. The newly synthesized progesterone represented 45% of total progesterone. A net synthesis of progesterone was found in the 4 ovaries which were perfused with medium containing no human chorionic gonadotropin indicating that gonadotropin as used in these experiments was not required for progesterone synthesis.

Studies on the radioactivity of the isolated progesterone in one of the experiments showed that the purified progesterone was radioactive as were the 2,4 dinitrophenylhydrazone derivatives. The incorporation of carbon-14 from 1-C^{14}-acetate into the carbon skeleton of progesterone in all of the experiments was less than 0.5% of the amount theoretically possible. This calculation is based on the premise that each molecule of progesterone would contain 10 carbon atoms derived from carbon-1 of acetate(12). If the C^{14}-labeled progesterone examined had been synthesized entirely from acetate-1-C^{14} present in the perfusate the specific activity of the synthesized progesterone would be 10 times that of the acetate on a molar basis. Low levels of radioactivity present in the isolated progesterone indicate that plasma acetate is not a major immediate precursor for the biosynthesis of progesterone of the corpus luteum in this system.

Our results show that an isolated luteal-phase ovary perfused with a blood substitute can synthesize progesterone and release it into the perfusate. The system can be used to study regulation of progesterone production and release into the blood. It may also have application in studies on the effects of drugs or toxic agents on progesterone production and release.

Summary. Corpora lutea of isolated luteal-phase swine ovaries perfused with a blood substitute synthesize progesterone without being stimulated by HCG. Synthesized progesterone was isolated by solvent extraction and thin layer chromatography. The isolated progesterone was identified by thin layer chromatography, gas chromatography, ultraviolet absorption spectra, and 2, 4 dinitrophenylhydrazone derivatives. Small but detectable amounts (less than 0.5% of the amount theoretically possible) of acetate-1-C^{14} were incorporated into the synthesized progesterone. Most of the progesterone (88% ± 12%) was found in the corpora lutea with the remainder in the perfusate. An average value of 43 μg of progesterone per gram of luteal tissue was synthesized during the perfusion period. Newly synthesized progesterone represented an average value of 45% of the total progesterone isolated from corpora lutea and perfusate.

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