TABLE IV. Comparison of Dog Blood Volumes Using $CdSO_4$-NaOH Precipitation (Method I) and Harington's Method (Method II).

Dog wt (kg)	Blood vol (ml) Method I	Method II	Method I (ml/kg)	Method I as % Method II
10.5	664	697	63	95.3
11.0	881	990	80	90.0
12.5	1,018	1,090	81	93.4
8.5	818	816	96	100.2
9.0	1,029	1,055	114	97.5
14.5	1,321	1,332	91	99.2

to incomplete adsorption on protein and precipitation of the dye, to a change in the light absorption characteristics due to the conditions of precipitation and extraction(3), or to impurities, in the sample of dye, which absorb light at 620 mμ under the conditions of the solution but either are or not adsorbed to protein and are colorless under these conditions. A failure of precipitation of a colored component is not tenable since by visual inspection the supernatant from the precipitation procedure is colorless. Information concerning the second two possibilities is not available. However, colored impurities present in commercial preparations of Evans' blue have been reported(4).

Table IV summarizes data from some determinations of the blood volume in dogs using the extraction procedure from plasma of Harington *et al*(5) and our precipitation procedure. The plasma volume values were calculated from a single blood sample withdrawn 5 minutes after the dye injection. Blood volumes determined simultaneously with the two methods average agreement within 5%.

Over a period of months we have measured the recovery of 0.01 mg/ml dye added to the plasma of different dogs and determined by different technicians using this procedure. In 25 experiments on different samples of plasma mean concentration and standard error were 0.0099 ± 0.00015 mg/ml.

Allen(6) introduced a paper pulp adsorption and extraction procedure for determination of Evans' blue in plasma which eliminates many of the objections inherent in reading the optical density of plasma directly. There have been numerous modifications of Allen's procedure. The procedure reported here utilizes the naturally occurring adsorption on plasma protein for the same purposes.

Summary. A relatively rapid procedure for determination of Evans' blue in plasma or in plasma diluted with dextran is described. The procedure involves the total precipitation of plasma proteins by $CdSO_4$ and NaOH. After centrifugation and acidification of the precipitate it is extracted with butanol. The O.D. of the extract, with ethanol added, is read at 620 mμ, and the dye concentration is read from a standard graph.

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In vitro Synthesis of Progesterone by Ovaries and Adrenals of Snakes.* (28971)

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Previous work has not established a relationship between the gonads and pregnancy maintenance in snakes. Clausen(1) found that castration of several species of a viviparous snake (Natrix) in early or mid-gestation caused resorption or abortion of the embryos,

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but in late gestation had little or no effect. However, attempts to maintain castrated Natrix with one mg of progesterone administered twice weekly for 3 weeks were unsuccessful. Bragdon(2) considered the effects of castration as limited to an interference with parturition.

Progestins have been demonstrated in the ovaries of certain crotalid snakes(3), and in the blood of Natrix by the Hooker-Forbes bioassay(4). Serum progestin concentration has been found to vary with the reproductive cycle and to increase during pregnancy; however, progestin was also found in the blood of male snakes. Identification of reptilian progestin and its endocrine source is an important step in an investigation concerning the physiological function of this steroid.

Materials and methods. Two genera of snakes, one oviparous (*Coluber c. constrictor*) and one viviparous (*Natrix sipedon pictiventris*) were obtained from a commercial supplier (Ray Singleton, Tampa, Fla.) between March and July of 1963. Animals were housed in a vivarium at 25°C in the laboratory. Snakes were anesthetised with Nembutal (30 mg/kg body weight)(5), the ovaries and adrenals removed, cleaned and cut into small pieces with a fine pair of scissors. The tissues were incubated in 10 ml Krebs-Hensleit Ringer's solution with 200 mg% glucose at pH 7.4. To each flask was added 0.017 μg ^{3}H-16-pregnenolone in 0.1 ml propylene glycol (giving 500,000 cpm) per 800 mg tissue. Incubation was carried out in a Dubnoff metabolic incubator in an atmosphere of 95% oxygen and 5% carbon dioxide at 24°C for 2 periods each of 4 hours' duration. Fresh media and precursor were added at the end of the first period after decanting the original medium. Pooled incubates from both 4 hour periods were frozen at —15°C until extraction.

Ovarian incubates were extracted as described by Short(6). Adrenal incubates were extracted 4 times with one volume of chloroform, the pooled extracts cooled for 30 minutes before washing with one-tenth volume 0.05 N NaOH followed by rinsing 5 times with one-tenth volume distilled water. The extract was evaporated to dryness on a rotary film evaporator at 45°C and then chromatographed.

Chromatography was performed in systems Bush A and Bush 5(7) and in the E2B and E4 systems of Eberlein and Bongiovanni(8). Reduction of progesterone was performed by the method of Warren and Salhanick(9) and acetylation as described by Bush(10). Radioactive spots corresponding to progesterone standards are located on paper using a Nuclear Chicago 4 pi scanner coupled to an integrating recorder (Texas Instruments Co.). Quantitation of results was performed using a Packard Liquid Scintillation Counter.

Results and comments. The presence of progesterone in incubates of ovarian and adrenal tissue from both species of snakes was suggested by the following criteria: (a) Comparison with authentic progesterone established identical Rf values in systems Bush A and E4. (b) On treatment with pyridine and acetic anhydride, the material failed to form an acetate. (c) Reduction of the material by treatment with sodium borohydride at 0°C gave a product with an Rf value identical to that of authentic 20 beta hydroxyprogesterone in system Bush A. (d) Treatment of the material corresponding to authentic 20 beta hydroxyprogesterone with pyridine and acetic anhydride produced a compound with Rf value identical to authentic 20 beta hydroxyprogesterone acetate in system Bush A. (e) A mixture of the isolated material and authentic progesterone was recrystallized 3 successive times from benzene/ethanol (1:1). The specific activity remained constant within the error of the measurements.

Since ovarian tissue from both snakes was able to synthesize progesterone *in vitro,* oviparity or viviparity does not appear to be related to ability to synthesize this steroid. Production of progesterone by adrenal tissue suggests the possibility that the adrenal may contribute to circulating progesterone *in vivo.* It is interesting to note that both the ovary and the adrenal of Natrix produce approximately 3 times more progesterone per unit tissue weight (3521 and 21, 427 dpm/g respectively) than the same organs of Coluber (1229 and 7481 dpm/g). Assignment of a

specific role for progesterone in the reproductive physiology of snakes must await conclusive identification of progesterone in peripheral blood and establishment of a relationship between the steroid and the reproductive process.

Summary. Ovaries and adrenals of both *Natrix sipedon pictiventris* and *Coluber c. constrictor* synthesized progesterone *in vitro* from ^{3}H-16-pregnenolone. Progesterone synthesis by the ovaries and adrenals of Natrix was 3 times that of Coluber.

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Dietary Bile Acids and Lipid Metabolism. IV. Dietary Level of Lithocholic Acid for Chicks. (28972)

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Several recent reports have demonstrated the hypercholesterolemic effect of dietary lithocholic acid in the chick(1-4). Previously, we have demonstrated that dietary lithocholic acid also markedly increases plasma lipid phosphorus levels and liver size(4,5). The increase in liver size has been shown to be the result of a bile duct proliferation(6) or the "ductular cell reaction"(7). These observations have recently been confirmed(3).

The effect of various levels of dietary lithocholic acid has not been studied. The data presented here were obtained in an effort to determine the minimal level of lithocholic acid required to produce the effects previously reported. In addition, the influence of dietary lithocholic acid on plasma β-lipoprotein levels has been studied.

Experimental. Male Hy-line White Leghorn chicks were employed in 2 experiments. In Exp. 1, the chicks were fed a stock diet for 7 days and in Exp. 2 for 10 days. At the end of this time, the chicks were allocated to the various experimental groups in such a way as to have almost identical initial mean body weights for all groups. In Exp. 1, 8 chicks were used per group with an average group weight of 65 or 66 g; 14 chicks were allocated to each group in Exp. 2 with an average group weight of 82 to 86 g.

The basal diet employed was identical to that used in previous studies(4). Lithocholic acid was added to the basal diet at the expense of glucose at the levels indicated in the Tables. The chicks were housed in heated cages having raised wire floors. Food and water were supplied *ad libitum.* Body weight and food consumption were determined at weekly intervals.

The experimental period in Exp. 1 was three weeks. In Exp. 2, half of each group was sacrificed after having received the experimental diets for 4 weeks; the remaining 7 chicks in each group were fed the diets for an additional 4 weeks. At termination of the study periods, blood was obtained by cardiac puncture with a heparinized syringe. The animals were sacrificed and the livers excised. After blotting to remove excess blood, the livers were weighed and a slice was fixed in 10% neutral buffered formalin. The remainder of the liver was frozen and stored at —20°C for chemical analysis.

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