arose from remaining parenchymal cells. There was a tendency to 2 or more cycles of cell proliferation and there was a tendency for cells in some areas to show more proliferation than other areas. Differences from mammals were a greater lag period between operation and DNA synthesis in amphibia; fewer cells showed DNA synthesis at any one time; and cell proliferation continued for a much longer time after partial hepatectomy.

It was evident from the rarity of mitotic figures as compared with the uptake of H^3-thymidine by liver cell nuclei that mitotic counts alone might give misleading findings regarding the relative importance of hypertrophy and hyperplasia in restoration of amphibian liver. The reasons for this discrepancy are not apparent; amitotic cell division might be a factor.

Summary. Regeneration was studied in the liver of the newt, *Triturus viridescens*, following approximately 78% partial hepatectomy. H^3-thymidine and autoradiographs were used to quantitate DNA synthesis in liver cell nuclei. DNA synthesis began 5 days after partial hepatectomy, reached a peak of 1.9% of liver cells at 10 days, fell to 0.2% at 20 days, rose to a second peak of 1.3% at 105 days, and continued at an elevated level of 0.5% at 150 days, the latest time studied. Liver weight was restored to normal 4 weeks after operation.

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Isolation of Dehydroepiandrosterone-C^{14} from Dogs Infused with Cholesterol-4-C^{14} by the Spermatic Artery.* (27767)

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Testosterone and androstenedione are secretory products of the canine testis(1,2). Dehydroepiandrosterone (DHEA), however, has only been isolated from porcine testicular tissue(3) and from rabbit testis slices incubated with acetate-1-C^{14}.† No report of its occurrence in spermatic vein blood has hitherto appeared. Since DHEA and DHEA acetate are converted to testosterone *in vitro*(4) and *in vivo*† it was considered important to establish whether the canine testis secretes this steroid into the blood stream.

Methods and results. The technics for obtaining spermatic vein blood from the left testis and of infusing radioactive compounds by the left spermatic artery of the anesthetized dog have been described(2,5).

Sixty ml of warm saline were saturated with cholesterol-4-C^{14}‡ and infused by the left spermatic artery into normal dogs at a con-

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† Hall, P. F., Sozer, C., Eik-Nes, K., submitted for publication, *Endocrinology*, 1962.

‡ Specific activity: 0.6 μc/mg.

stant rate for 90 minutes. Spermatic vein blood was collected in 30-minute samples during infusion. The plasma was separated from red cells by centrifugation, the cellular layer washed with saline and the combined plasma and saline wash extracted 4 times with 1.5 volumes ethyl acetate:ether (1:1) each time. The extract was washed twice with 0.1 volume 1 N sodium hydroxide, and twice with 0.5 volume water and evaporated to dryness under nitrogen. The residue was dissolved in 15 ml 70% aqueous methanol, kept at 5°C for 12 hours, centrifuged in the cold and the methanol decanted(6). Precipitated fat was washed twice with ice cold 70% methanol, the combined methanol fractions were dried under nitrogen until the methanol was evaporated, and the aqueous phase was extracted twice with 50 ml ether. This extract was evaporated to dryness under nitrogen and the residue chromatographed on paper in methylcyclohexane/propylene glycol(7).

The chromatograms were scanned in a thin window Geiger strip counter. Two distinct peaks of radioactivity were found: one at the front of the chromatogram and one at the origin. The material at the front was eluted and subjected to gas-liquid chromatography. On a 6-foot glass column(8) it had a retention time identical to authentic cholesterol chromatographed on the same column on the same day. The radioactive material near the origin was eluted, acetylated(9) and rechromatographed on paper in the same system with testosterone-4-C^{14} acetate, DHEA-4-C^{14} acetate, and androstenedione-4-C^{14} run on a reference strip. In the plasma extracts from each of 4 dogs, radioactive material behaving chromatographically like all 3 of the standard compounds was found. Most of this radioactivity ran like testosterone acetate. Since testosterone and androstenedione have been adequately isolated and identified from canine spermatic vein blood(1,2), no further attention was paid to these steroids in the present investigation. Radioactive material behaving like DHEA-4-C^{14} acetate on the reference strip was eluted and the eluates from 4 dogs were pooled and rerun in the same system of paper chromatography. The radioactivity appeared as a single peak behaving like authentic DHEA-4-C^{14} acetate. This peak was eluted, the eluate divided in 2 aliquots, to one of which 50 mg carrier DHEA acetate was added and the mixture crystallized to constant specific activity (Table I). The other aliquot was dissolved in 1 ml 0.1 N ethanolic sodium hydroxide and incubated at 37°C for 1 hour. It was then diluted with 5 ml water, the ethanol evaporated under nitrogen and the aqueous layer extracted with ethyl acetate:ether (1:1, v/v). The extract was evaporated to dryness and chromatographed on paper in methylcyclohexane/propylene glycol with DHEA-4-C^{14} acetate and DHEA-4-C^{14}§ on a reference strip. The radioactivity of the saponified material now behaved like authentic DHEA-4-C^{14} and was eluted, mixed with 50 mg carrier DHEA and crystallized to constant specific activity (Table II).

TABLE I. Recrystallization of Plasma DHEA-C^{14} Acetate to Constant Specific Activity.*

Recrystallization	Solvent	Specific activity (dpm/mg)
Original mixture	Acetone	141
1st	Methanol	129
2nd	Benzene	126
3rd	Petroleum ether	130
4th	Ethanol	121

* Counted in a Packard automatic liquid scintillation spectrometer using oxygen-free toluene-POP-POPOP scintillation solvent. Counting efficiency was 55%.

TABLE II. Recrystallization of Plasma DHEA-C^{14} to Constant Specific Activity.*

Recrystallization	Solvent	Specific activity (dpm/mg)
Original mixture	Acetone	110
1st	Methanol	96
2nd	Petroleum ether	98
3rd	Ethanol	91
4th	Benzene	90

* Counted as indicated in Table I.

Immediately following infusion of the spermatic artery with cholesterol-4-C^{14}, 250 ml blood were collected from the jugular vein in all animals. When the plasma was processed as outlined for spermatic vein plasma, DHEA-C^{14} acetate or DHEA-C^{14} could not be found.

§ Obtained by saponification of DHEA-4-C^{14} acetate.

Thus conversion of cholesterol-4-C^{14} to DHEA-C^{14} must have taken place in the testicular tissue.

Discussion. The present findings show that the dog testis is able to convert cholesterol to DHEA and to secrete this steroid into its venous blood. It is unlikely that the DHEA isolated represents testicular metabolism of androstenedione or testosterone, since when these steroids labeled with C^{14} in position 4 were infused by the spermatic artery, no DHEA-C^{14} could be detected in spermatic vein blood.† Furthermore, administration of radioactive testosterone to human subjects will not result in excretion of radioactive DHEA in the urine(10).

The data of the present investigation, therefore, together with previous observations† (3), strongly suggest that DHEA is both produced and secreted by dog testicular tissue. Since the conversion of cholesterol to DHEA was lower than to testosterone or even addrostenedione(2) in all animals, it is at present difficult to assign biological importance to testicular secretion of DHEA in the dog. Furthermore, DHEA possesses only *ca.* 1/20 of the androgenicity of testosterone. It is possible that during synthesis of testosterone, where DHEA can serve as an intermediate† (3,4,11), excess DHEA may simply leak out from the interstitial cell and escape by the spermatic vein blood.

Summary. Dehydroepiandrosterone-C^{14} has been isolated in spermatic vein blood of anesthetized dogs infused with cholesterol-4-C^{14} by the spermatic artery. It is considered that this finding lends additional support to the view that dehydroepiandrosterone is an intermediate in the biosynthesis of testicular androgens.

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Properties of Serum Fractions Derived from Wheal Reactive Diphtheria Antitoxin.*† (27768)

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Allergic reagins or skin sensitizing antibodies possess the unique property of inducing wheal reactions following combination in skin with the corresponding antigen. This property may be recovered in serum fractions, although its distribution in these fractions is different from that of non-sensitizing antibodies. For example, skin sensitizing antibodies migrate electrophoretically as γ_1 globulins in contrast to non-sensitizing precipitating antibodies which migrate as γ_2 globulins (1,2,3,4). It is unfortunate that attempts to utilize such methods as zone electrophoresis,

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