

Presence of Progesterone in Extracts of Ovaries of Laying Hens.* (23001)

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The post-ovulatory history of ovarian follicles in birds differs from that in mammals; and there does not appear to be any structure in the bird analogous to the mammalian corpus luteum. However, extracts of avian blood have been shown to have progestogenic activity(1), as assayed by the Hooker-Forbes (2) test, and various experiments on effects of exogenous progesterone, administered either alone or in conjunction with estrogen, suggest that progesterone, or hormone of equivalent effect, is produced in the laying hen and is important in its reproductive function(3). We have obtained chromatographic and spectrometric evidence for the presence of progesterone in ovarian follicles of laying hens.

Methods. Ovaries were removed from 25 laying hens immediately on killing. The large yellow ova were at once removed from their follicles and discarded and the residual ovarian tissue and follicles were disintegrated in methanol-ether (3:1) in a Waring blender. Extraction of steroid material was carried out as described by Edgar(4). The final extract was chromatographed on filter paper (Whatman No. 1) by use of ligroin—80% methanol system(5). Examination of the paper under ultraviolet hand lamp revealed a prominent absorbing spot at the R_f realized for pure progesterone under similar conditions. The spot reacted positively to 2, 4-dinitrophenylhydrazine. The spot was eluted from the paper with ethanol and the ultraviolet absorption spectrum of the eluate determined on a recording spectrometer (Warren Spectracord as shown in Fig. 1, curve A).

Results. The eluted material was rechromatographed in the same system. No change in R_f value was observed, but the eluate from the second chromatogram gave the absorption spectrum shown in Fig. 1, curve B.

Fig. 2 shows absorption spectra obtained

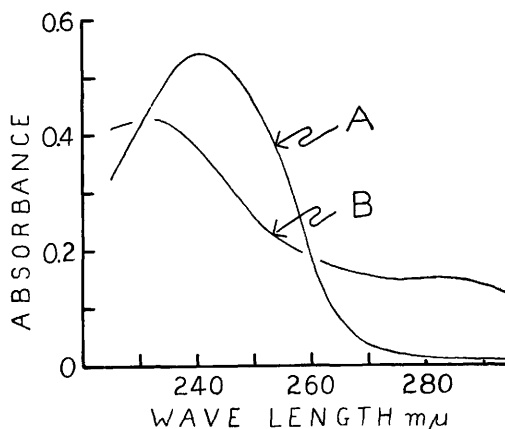


FIG. 1. (A) UV-absorption spectrum of eluates of spot on chromatogram of extract of ovaries of laying hens. (B) UV-absorption spectrum of same spot after previous spectrometry and re-chromatographing.

from a pure sample of progesterone (100 γ) after each of 3 successive chromatographic runs under conditions similar to those used to separate the ovarian extract. Curve A, Fig. 1 is similar to curve A, Fig. 2, while curve B, Fig. 1 and curve C, Fig. 2 show close correspondence in detail. Savard *et al.* (6) have presented evidence that progester-

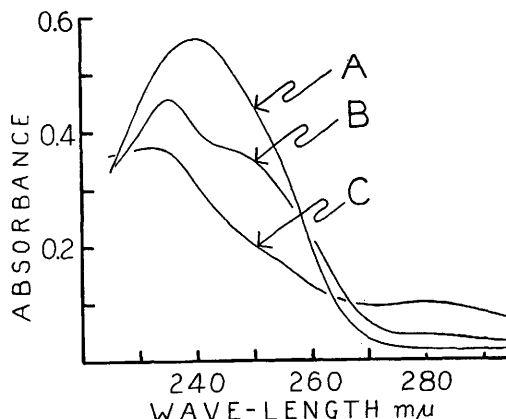


FIG. 2. (A) UV-absorption spectrum of reference progesterone after single chromatographic run. (B) Absorption spectrum after re-chromatography. (C) Absorption spectrum after further re-chromatography.

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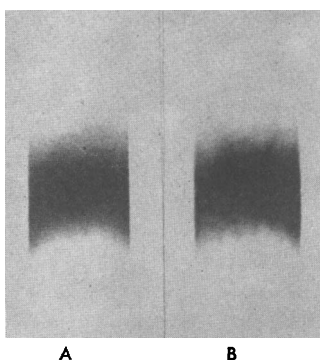


FIG. 3. (A) Spot on chromatogram carrying tracer amount of $^{21}\text{-C}^{14}$ -labelled progesterone; stained with 2,4-dinitrophenylhydrazine and photographed using blue filter. (B) Radioautograph of same spot. (The photographs do not fully reproduce the detail of correspondence.)

one is broken down on filter paper by exposure to ultraviolet light. The absorption spectrum of the breakdown product is closely similar to that shown in curve B, Fig. 1, and curve C, Fig. 2. These results were interpreted as evidence that the compound isolated from avian ovaries was progesterone, and that changes in the absorption spectrum were due to chemical breakdown of progesterone under conditions of chromatography and ultraviolet spectrophotometry.

Another sample of the ovarian compound was mixed with 5 γ of $^{21}\text{-C}^{14}$ -labelled progesterone and carefully rechromatographed without having been exposed to UV spectrophotometry. The strip was dried and then left in contact with X-ray film for 4 days to secure a radioautograph. The spot was then stained with 2, 4-dinitrophenylhydrazine. Under our conditions the 5 γ of tracer progesterone gave a barely perceptible stain, whereas the labelled spot stained strongly. It will be seen from Fig. 3 that the radioautograph and staining of the spot showed close correspondence. This observation provides

strong additional evidence for identity of the ovarian compound with progesterone.

We have failed to detect progesterone in blood of laying hens. However, extracts prepared by the method of Edgar(4) from 2 litres of blood from laying hens contained material which has an absorption spectrum similar to those shown in Fig. 1, curve B, and Fig. 2, curve C.

In other experiments the progesterone spot has been found on chromatograms of extracts of maturing follicles as well as of ruptured follicles. Although our results are mainly of qualitative significance, the degree of staining has suggested that the quantities of progesterone in the ovaries or follicles were small.

Summary. Progesterone has been detected on chromatograms of extracts of ovaries of laying hens and identification has been confirmed by radioautographic fingerprinting.

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