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Studies on the Anterior Pituitary Hormones of the Kangaroo[1] (37947)

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(Introduced by Choh Hao Li)
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The body of existent knowledge on the anterior pituitary hormones has been largely derived from studies on the eutherian mammals, while almost nothing is known about methatherian (marsupial) mammals. We report here studies on a small quantity of kangaroo (*Megaleia rufa*) pituitaries, which were fractionated by a scheme designed to obtain most of the known hormonal activities of the anterior pituitary. Fractions containing follicle-stimulating hormone (FSH), luteinizing hormone (LH, ICSH), growth hormone (GH), prolactin (PRL), and adrenocorticotropin (ACTH) activity were identified, partially purified, and compared to well-characterized mammalian pituitary hormones.

Fractionation of kangaroo pituitaries. The glands were obtained by Dr. Donald S. Bradshaw (Department of Zoology, University of West Australia, Nedlands) and put at our disposal by Dr. Paul Licht (University of California, Berkeley). Two batches were employed, the first consisting of 2.3 g acetone-dried pituitaries which were several years old, and a second batch of 0.73 g which was collected and acetone dried in the preceding year. Both batches were handled identically and the fractions of each were combined for further purification after comparable results were obtained with each. The fractionation procedures were adapted from methods developed in this laboratory for the purification of individual mammalian pituitary hormones (1). In brief, the glands were homogenized in a Waring Blender with cold water, adjusted to pH 9.5 with $Ca(OH)_2$, and stirred at 4° for 3 hr. After centrifugation, the alkaline extract was fractionated with $(NH_4)_2SO_4$, which was initially added to a concentration of 0.5 saturation (0.5 SAS); a second fraction (0.5–0.8 SAS) was obtained by adding $(NH_4)_2SO_4$ to the 0.5 SAS supernatant fluid to a final concentration of 0.8 SAS. The 0.5 SAS fraction was dialyzed, lyophilized, and extracted with phosphate buffer, pH 5.1, 12% SAS. After removal of the insoluble material (fraction P), the extract was chromatographed on Amberlite IRC-50 as previously described (2, 3) and yielded four fractions: the initial pH 5.1 unadsorbed fraction (A), a water eluate (B), pH 6.0 eluate (C), and an alkaline (pH 9.5) eluate (D). The 0.5–0.8 SAS fraction was further purified by extraction with 10% NH_4Ac, pH 5.1, 40% EtOH, a technique known to extract glycoproteins (4). This fraction was designated SS. The procedure was also applied to the Amberlite IRC-50 fraction A, yielding fraction AS. The residue (R) remaining after the initial alkaline extraction and the P fraction were each extracted with an acid–acetone mixture routinely employed in this laboratory to prepare ovine prolactin and ACTH (1), dialyzed, and lyophilized.

These materials were the major fractions initially tested for different hormonal activities. LH activity was determined by the ovarian ascorbic acid depletion (OAAD) test (5) and FSH by the Steelman–Pohley assay (6). GH fractions were identified by precipitin reactions in agar gel (7) using

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monkey antiserum to purified rat GH (8) and by the rat tibia test (9). Prolactin was identified electrophoretically using polyacrylamide disc electrophoresis (10), by precipitin reactions in agar gel using rabbit antiserum against ovine prolactin, and assayed by the pigeon crop sac test (11). ACTH was assayed by an *in vivo* adrenal steroidogenic assay (12). Standard gonadotropin preparations were obtained from the Endocrinology Study Section of the N.I.H.

These assays revealed LH and GH activity in the C fraction, FSH activity in SS, prolactin in P and R, and ACTH in R. Fractions AS, B, and D were without significant activity. Interestingly, with human pituitaries fraction B contains growth hormone (1). The further purification and characterization of several of the hormonally active fractions will be described below.

Results and Discussion. Gonadotropins. The results of bioassays for LH and FSH obtained with the major fractions described above are shown in Table I. The % total activity distribution clearly shows that the majority (86%) of the LH activity is in the C fraction while the FSH activity (89%) is in SS. These results showing the separation of kangaroo LH and FSH by $(NH_4)_2SO_4$ precipitation parallel those found with many species of mammalian pituitaries (13). In addition, purified mammalian LH (ovine, porcine), like the kangaroo LH here, also elutes in fraction C on Amberlite IRC-50 chromatography (unpublished studies). Also, it may be inferred that the kangaroo gonadotropins are glycoproteins since they are soluble in the NH_4Ac–EtOH extraction technique. Thus, in these respects, the kangaroo gonadotropins resemble the mammalian hormones.

Further purification of kangaroo FSH was achieved by chromatography on sulfoethyl-Sephadex C50 in a manner similar to that described for ovine FSH (14). Figure 1 shows the results obtained on chromatographing the SS fraction. The active eluate, the 0.03 *M* NH_4HCO_3 peak, had a potency of 2.7 × N1H-FSH-S1 and could be additionally purified by gel filtration on Sephadex G-100 (Fig. 1). The B fraction from the experiment shown in Fig. 1 was found to have an activity of 19.4 × N1H-FSH-S1. Highly purified ovine FSH preparations obtained by similar techniques have had activities of about 50 × N1H-FSH-S1 (14).

The Amberlite IRC-50 fraction C, containing both LH and GH activity, was chromatographed on DEAE-cellulose in 0.03 *M* NH_4HCO_3, pH 9.0. With a variety of analogous mammalian and nonmammalian fractions chromatographed under these conditions, we have found that the unadsorbed fraction (DEAE-A) contains the LH, and the adsorbed GH can be eluted with 1 *M* NH_4HCO_3 (in preparation for publication). Attempts, however, to further purify kangaroo LH by this procedure were unsuccessful, due perhaps to the technical difficulties encountered in handling very small quantities of material.

Growth hormone. The DEAE-B fraction, containing the kangaroo growth hormone activity, was applied to a small Sephadex G-100 column, in 0.05 *M* NH_4HCO_3. Three major elution peaks were pooled, corresponding to *Ve/Vo*'s of approximately 1–2

TABLE I. Fractionation of Kangaroo Gonadotropins by $(NH_4)_2SO_4$ Precipitation and Amberlite IRC-50 Chromatography.

Fraction	Yield[a] (mg)		Potency[b] (U/mg)	Total activity U	Total activity %
AS	10	LH	0.04	0.4	9
		FSH	0.56	5.6	11
C	27	LH	0.14	3.8	86
		FSH	—	—	—
SS	23	LH	0.01	0.2	5
		FSH	1.90	43.6	89

[a] Obtained from 725 mg dry weight of pituitaries. See text for details.

[b] Potencies are expressed in terms of NIH-LH-S1 and NIH-FSH-S1.

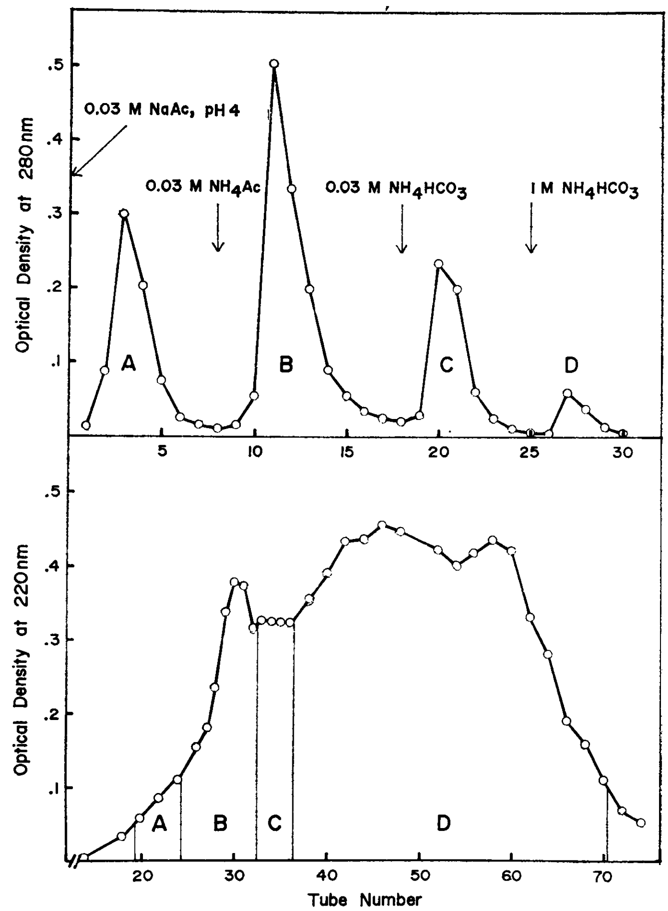

FIG. 1. *Upper*. Chromatography of 22 mg of kangaroo FSH fraction SS on a 10-ml column of sulfoethyl-Sephadex C50; 5 ml/tube; FSH activity was located in peak C.

Lower. Gel filtration of the active FSH fraction obtained above (peak C) on a 130-ml column of Sephadex G-100 in 0.05 *M* NH_4HCO_3; 3.3 mg applied; 1.5 ml/tube; FSH activity is mainly in peak B.

(A), 2–4 (B), and 4–6 (C). These fractions were tested for the presence of GH by the Ouchterlony technique (7) using monkey antiserum against rat GH. The results indicated that GH was mainly in peak A, but detectable in peak B as well. The results with the A peak are shown in Fig. 2A. It can be seen that kangaroo GH gives a reaction of complete identity with rat, bovine, and porcine GH. The B peak gave similar results and both were active in the rat tibia test, the A peak resulting in a tibial cartilage plate width of 215 nm and B of 203 nm when assayed at a dose of 100 μg.

Prolactin and ACTH. A fast migrating band characteristic of prolactins (10) was observed when the P fraction was examined by disc electrophoresis. In addition, a similar but fainter band was observed when the residue was examined. On this basis, these fractions were pooled, dissolved in water, and adjusted to pH 5.3 to obtain a precipitate. The disc electrophoresis pattern of this precipitate is shown in Fig. 3 and it can be seen that there is one major staining band, a very fast migrating one. This evidence, suggesting the presence of prolactin, was confirmed by data from the pigeon crop sac assay which showed the preparation to have a potency of 0.11 × N1H-O-PRL.

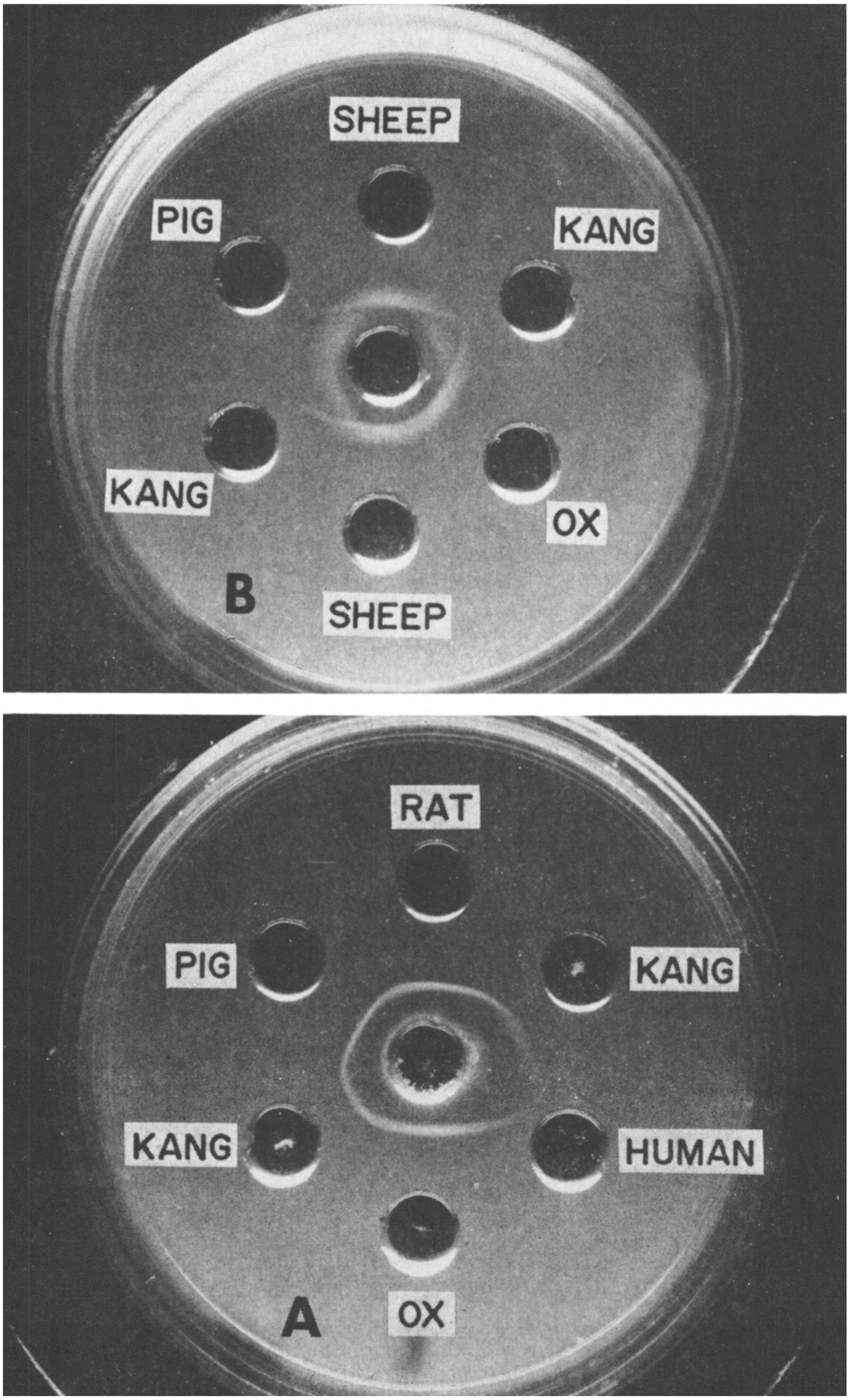

FIG. 2. (A) Ouchterlony plate showing the reaction of a monkey antiserum to rat growth hormone (center well, 0.1 ml) with purified kangaroo, pig, rat, and ox GH (20 μg each). Note reaction of complete identity and lack of reaction of human GH.

(B) Ouchterlony plate showing reaction of rabbit antiserum to ovine prolactin (center well, 0.1 ml) with 40 μg each of kangaroo, pig, ovine, and ox prolactin. Note reaction of identity between kangaroo and pig prolactin and/or partial identity with ovine and ox prolactin.

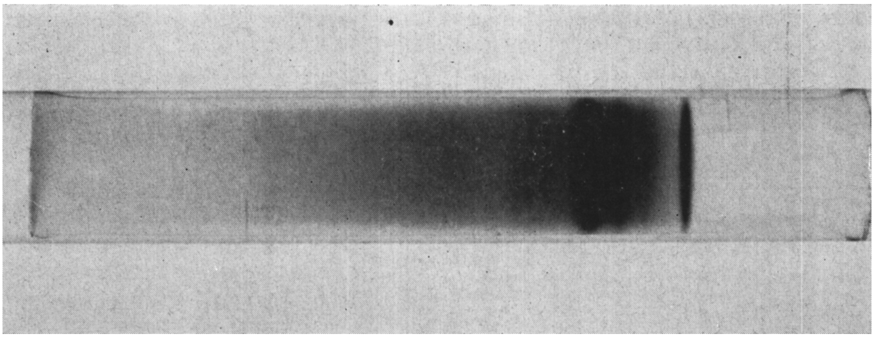

FIG. 3. Disc electrophoresis pattern of kangaroo prolactin; pH 8.3, 7% gel; stained with Amido Schwartz; migration from left to right; prolactin bands located just before right-most dye front band.

Figure 2B shows the results obtained by the immunological precipitin test in agar. It is seen that the kangaroo PRL cross-reacts with the antiserum against ovine PRL but forms a line of incomplete identity. Interestingly, porcine PRL reacts in an identical manner. It may be concluded that porcine and kangaroo PRL are immunochemically more closely related than to ovine or bovine PRL, but all four share common immunological determinants.

ACTH was only briefly examined. Activity was found in the AAP from the residue amounting to 3 U/mg. Other fractions were not tested and the active material was not further purified.

Concluding remarks. Aside from a brief report (15) describing the extraction of gonadotropins from pituitaries of the Tammar Wallaby, there is virtually no information existent on the biochemical nature of the marsupial pituitary hormones. In these studies, all the major mammalian anterior pituitary hormones were located in the kangaroo pituitary extracts and in most cases purified to some extent. While fractions were not examined for the presence of thyrotropin in these studies, it seems reasonable to expect on the basis of our results that it is also present. The kangaroo gonadotropins, FSH and LH, and GH followed mammalian hormone behavior in $(NH_4)_2SO_4$ fractionation and Amberlite IRC-50 chromatography, suggesting chemical similarity. Further evidence of the chemical relatedness of kangaroo FSH to mammalian FSH is demonstrated by its solubility in NH_4AC–EtOH solution and the elution pattern (Fig. 1) obtained by sulfoethyl-Sephadex chromatography. Kangaroo GH and prolactin are also seen to be related to their mammalian counterparts by the immunochemical studies (Fig. 2). There were insufficient quantities of any of the purified materials to do additional tests for chemical identity such as terminal-group or amino-acid analyses. The bioassays provided evidence of biological similarity to mammalian hormones although the potencies observed were low. While this may reflect a lack of final purity, it could also reflect species specificity in terms of the marsupial hormones interacting in the eutherian mammalian and avian assays. Our results suggest most of the kangaroo pituitary hormones can be separated and further purified by the procedures outlined here.

Summary. A small quantity of kangaroo pituitaries were subjected to fractionation procedures known to separate mammalian pituitary hormones. These involved $(NH_4)_2SO_4$ fractionation, ion-exchange chromatography, and gel filtration. The results show that the kangaroo possesses pituitary hormones which behave very much like their mammalian counterparts with respect to biological, chemical, and immunochemical behavior.

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