HISTOLOGICAL AND BIOCHEMICAL STUDIES ON THE OVARY AND OF PROGESTERONE LEVELS IN THE SYSTEMIC BLOOD OF THE GREEN ACOUCCHI (*MYOPROCTA PRATTI*)

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Summary. Combined histological and biochemical studies have been made in a small series of pregnant and non-pregnant acouchis. All pregnant animals had twin foetuses and their ovaries contained two to five large corpora lutea (CL) of ovulation and variable numbers of accessory CL which were very much smaller in size and bore evidence of having arisen from un-ovulated follicles. Normal Graafian follicles were also present, together with many small atretic follicles.

The true corpora reached maximum size by the end of the 2nd week of pregnancy and had regressed before parturition. The occurrence of maximum secretory activity in early pregnancy, as indicated from estimates of the mean size of luteal cells, was confirmed by assays of luteal progesterone concentration.

The accessory CL tended to increase in number as pregnancy advanced. The luteal cells, though slightly smaller, were otherwise indistinguishable from those of the true corpora and, depending on the stage of pregnancy, contributed 25 to 100% of the total amount of progesterone secreted by the ovary.

In early pregnancy, plasma progesterone concentration increased rapidly to a level that was four times as great as that found in non-pregnant acouchis, and which thereafter declined.

The rate of growth and decline of the CL of pregnancy, their secretory activity and the levels of progesterone in the systemic blood are compared with those reported in some other hystricomorph rodents.

INTRODUCTION

The green acouchi is a rare hystricomorph rodent which inhabits the tropical forests of the north-western region of South America. It is small in comparison with some other species of this sub-order of rodents (adults weigh about 1 to 1.25 kg), and is of slender build. It has long thin legs which afford great

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agility of movement and a very short slender tail which vibrates rapidly and, according to Morris (1962), is used as an important sexual signalling device.

This acouchi has been bred in the Zoological Society of London in Regent's Park and detailed observations have been made on its feeding and hoarding habits (Lyall-Watson, 1964). Very little information has been published on the anatomy and physiology of its reproductive organs, apart from some observations made by Weir (1967); these included the presence of a vaginal closure membrane and numerous accessory corpora lutea (CL) formed from un-ovulated follicles, and the length of the oestrous cycle.

A breeding colony was established for a detailed study of reproductive behaviour in this species (Kleiman, 1969) and to obtain basic information on its breeding potential in captivity (Kleiman, in preparation). Some of these animals and their progeny were used in the present investigation of ovarian changes and progesterone secretion during pregnancy for comparison with similar studies being undertaken at this Institute on a variety of allied species, as a contribution to our understanding of the endocrinology of gestation in mammals.

MATERIALS AND METHODS

Animals

Ten acouchis derived from the colony studied by Kleiman (1969) were used for this investigation. They included three non-pregnant animals that had been observed previously by Dr B. J. Weir in this Institute who kindly supplied material from them for histological investigation. The seven remaining animals had been mated to provide a specimen at about fortnightly intervals throughout gestation (100 days); all except one (AF 16) had conceived. Data relating to age, weight and reproductive condition of these animals are summarized in Table 1.

Collection of specimens

The animals were anaesthetized surgically by intraperitoneal injection of Nembutal (Abbott) at the rate of 40 mg/kg and a sample of blood not exceeding 25 ml was withdrawn from the left ventricle into a syringe containing heparin. The plasma was separated and stored at -20°C .

The animals were then killed with ether and the ovaries were removed and examined. The ovary that contained one or more large CL was selected for biochemical study. After dissection, these CL were weighed individually (to 0.1 mg) on a torsion balance, pooled in cold ethanol and stored at -20°C . Except in one animal (AF 20), no attempt was made to dissect any of the numerous small accessory CL and they were included in the residual ovarian tissue which was weighed and preserved, as were the larger CL.

The remaining ovary and reproductive tract, the pituitary, thyroids and adrenals were fixed in Bouin's fluid and the embryos in 5% formol-saline. All tissues, other than the embryos, were weighed before and after fixation and paraffin sections were cut at 5 μ . The ovaries were cut serially, mounted completely (or every tenth section) and stained in H and E.

TABLE 1
SUMMARY OF BREEDING HISTORY, REPRODUCTIVE CONDITION AT AUTOPSY AND WEIGHT OF ORGANS OF ACOUCHIS

Acoushi no. (AF)	Age (years)	No. of previous litters	Condition at autopsy	Body weight (kg)	Fixed weight of organs (mg)						No. of true CL
					Ovary	Thyroids	Adrenals		Pituitary		
							Left	Right			
6	3 $\frac{11}{16}$ approx.	3	20 P	1.255	51.0	43.0	124	108	25.0	2	
17	1 $\frac{1}{16}$	0	29 P	0.982	14.8	26.3	216			16.4	2
12	2	2	45 P	1.163	48.7	40.1				83	63
15	1 $\frac{4}{16}$	1 (Ab)	60 P	1.108	83.5	37.5	153	133	27.2	2	
1	4 $\frac{1}{16}$ approx.	5+	75 P	1.333	64.5	32.1	92	93	19.7	5	
20	1 $\frac{1}{16}$	0	90 P	1.082	37.2	32.0	163	143	22.8	0	
13	3 approx.	4	5 p.p.	1.050	27.6	57.2*	145	130	26.0	0	
14	2 $\frac{9}{16}$	4	10 L	1.030	53.7	46.6	91	89	—	0	
16	1 $\frac{1}{16}$	2	21 L; 15 p.c.	1.072	39.0	40.0	226	178	18.6	2	
21	1 $\frac{1}{16}$	0	4 V	0.860	6.6	24.8*	75	57	17.2	1	

P = days pregnant; p.p. = days post partum; p.c. = days post coitum (infertile); L = days lactating; V = days from onset of vaginal opening; Ab = aborted; * = weight of one lobe $\times 2$.

Quantitative histological procedures

Estimates of the size of the CL of ovulation in pregnant and non-pregnant animals were obtained from measurements made of three diameters as described by Rowlands & Heap (1966). The accessory CL were counted by projecting ovarian sections from five or six levels on to a screen and the average number per section was recorded. Only the largest accessory CL were measured.

At least twenty lutein cells and their nuclei were measured in one or more of the two types of CL. Cells having a well-defined outline and a nucleus containing a prominent nucleolus were selected for this purpose.

Solvents and reagents used in biochemical studies

Solvents and reagents, other than 20 β -hydroxysteroid dehydrogenase type II and β -NADH (Sigma Chemical Co.), were purified by redistillation crystallization or thin-layer chromatography (TLC) in the laboratory before use. These included benzene, tetrahydrofuran (Hopkin and Williams Ltd), monochloroacetic anhydride (BDH), steroids (Steraloids Ltd) and [4-¹⁴C]progesterone (Radiochemical Centre, Amersham).

TABLE 2
SPECIFIC ACTIVITY OF POOLED 20 β -HYDROXYPREGN-4-EN-3-ONE
MONOCHLOROACETATE DERIVED FROM ACOUCHI PLASMA AND
OVARIAN TISSUES, EXPRESSED AS MC/MM

TLC purification	Sources		
	Plasma	Luteal tissue	Residual tissue
Benzene : ethylacetate (6 : 1)	4.90	2.30	4.62
Benzene : ethylacetate (6 : 1)	4.14	2.58	5.32
Benzene : ethylacetate (4 : 1)	2.97*	2.06	4.25

* The chloroacetate showed signs of hydrolysis and probably self-radiolysis at this stage. A peak having the retention time of 20 β -hydroxypregn-4-en-3-one appeared on the GLC chromatogram.

Summary of biochemical methods

The method used for the quantitative determination of progesterone was similar to that described by van der Molen & Groen (1965). It involved an initial purification of the crude extract by TLC, enzymatic reduction of progesterone to 20 β -hydroxypregn-4-en-3-one, and the transformation of the latter to its monochloroacetate derivative (Landowne & Lipsky, 1963; Brownie, van der Molen, Nishizawa & Eik-Nes, 1964) and finally quantified by gas-liquid chromatography (GLC) with electron capture detection. [4-¹⁴C]Progesterone (specific activity 58.5 mc/mm) and testosterone monochloroacetate were used as internal standards.

The precision of this method is well established for the determination of progesterone and testosterone in plasma but not for progesterone from tissue sources. Five precision experiments were performed, therefore, by assaying known amounts of progesterone (100 to 2500 ng) added to guinea-pig ovarian tissue (50 mg) from which all endogenous progesterone had been extracted. The

TABLE 3
RELATION BETWEEN THE NUMBERS OF CL AND EMBRYOS OF THE ACOUCHI

Acouchi no. (AF)	Condition at autopsy	CL of ovulation			Embryos			Ave. no. acc. CL /section	Diam. of largest follicle (mm)
		In fixed ovary No.	In dissected ovary No.	Wt (mg)	Lt ut.	No.	CR* length (mm)		
6	20 P	1	1 (Lt)	6.3	1	1	—	4.5	0.65
17	29 P	0	2 (Rt)	—	0	2	—	6	0.65
12	45 P	(3) ?	2 (Rt)	0.4	0	2	61 ♂	49	0.60
15	60 P	1	1 (Lt)	1.9	1	1 (Re)	59 ♀	59	0.50
1	75 P	1	4 (Rt)	1.7	0	2	94 ♂	12	1.00
20	90 P	0	0 (Lt)	—	2	0	136 ♀	51	0.70
							142 ♀		
							168 ♀		
							151 ♂		
14	10 L	0	0	—	0	0	—	20	0.50
16	21 L; 15 p.c.	0	2 (Lt)	—	0	0	—	61	0.55
21	4 V	0	1 (Rt)	—	—	0	—	4	0.0

Re = resorbing; other abbreviations as in Table 1.

* CR = crown-rump.

percentage error was found to be 11.10. Full details of the method used will be published elsewhere (Tam, in preparation).

For further characterization of progesterone, and because of the low titre found, the remaining progesterone derivatives from similar sources were pooled after GLC and further purified by TLC. The specific activity of the samples was then determined in each step. Table 2 shows that specific activity does not change within the limits of the experimental conditions.

RESULTS

General observations

Some quantitative information recorded at the time of examination is presented in Table 1. Body and ovarian weight are very variable and the latter bears little relationship to the number of CL of ovulation. The ovulation number varied between two and five and the resulting CL were recognizable macroscopically in all except one animal (AF 20) which was near to term. The left adrenal was found to be consistently heavier than the right but there was no evidence of an increase in either adrenal in the late stages of pregnancy as seen in the guinea-pig.

The uteri of all pregnant animals contained twin fetuses, all of which were healthy except one of the pair in AF 15 killed on the 60th day; this animal aborted her previous litter. The number of CL of pregnancy exceeded that of embryos in two specimens (AF 12 and 1) which suggests the occurrence of some embryonic loss. The initial rate of embryonic growth in the acouchi would seem to be very slow but is greatly accelerated in the later stages of gestation and, in this respect, is typical of some other hystricomorph rodents, e.g. the coypu, *Myocastor coypus* (Newson, 1966). The available data showed embryo age to be linearly related to the cube-root of embryo weight after the 30th day (gestation = 100 days). In coypus, a similar relationship is held from the 55th day (gestation = 132 days).

Corpora lutea

The presence of accessory CL, formed by luteinization of the membrana granulosa of un-ovulated follicles in the ovary of the adult acouchi as reported by Weir (1967), is confirmed. These structures were distinguished from the true CL by their very much smaller size and the presence of a degenerating egg or crumpled remains of a zona pellucida. Difficulty in classification arose only in the case of three corpora of intermediate size in one animal (see below).

EXPLANATION OF PLATE 1

FIG. 1. Ovary of acouchi (AF 6) containing a CL of pregnancy (20 days) with extravasated blood core, three vesicular follicles and three small accessory CL. $\times 18$.

FIG. 2. Graafian follicle of acouchi (AF 17, 29th day of pregnancy) showing well-developed theca interna. $\times 100$.

FIG. 3. Early development of accessory CL in AF 1 on 75th day of pregnancy with retained egg and a narrow peripheral ring of basophilic granulosa cells. $\times 88$.

FIG. 4. Fully formed accessory CL in AF 1 showing egg remnant and persistence of the ring of granulosa cells. $\times 125$.

PLATE 1

(Facing p. 538)

PLATE 2

(Facing p. 539)

Quantitative data concerning the corpora lutea of ovulation and the accessory corpora lutea are given in Table 3.

Corpora lutea of ovulation. Very large CL occurred in the acouchi after mating. Those recovered from the dissected ovary of four animals up to 45 days of gestation were pink in colour and had an average weight of 9.2 mg; the largest occurred in a non-pregnant acouchi (AF 16) on the 15th day after mating. The fixed ovary of only one (AF 6) of these four animals contained a CL of comparable size. Another animal (AF 12) had three CL of ovulation which were much smaller than the above, but were considerably larger than accessory corpora present in this or any ovary examined histologically.

A central core of extravasated blood was found in the true corpora during the initial period of their growth (see Pl. 1, Fig. 1). There was some evidence of a reduction in the size of the CL of pregnancy during the second half of gestation and of a complete regression during the period shortly before parturition (see AF 20); during ageing, their colour changed to light yellow. However, one of the ovaries of AF 14, killed 10 days *post partum* (suckling one young) contained one greatly regressed CL of pregnancy. A *post-partum* ovulation had not occurred in this animal or in AF 13. In one non-parous, unmated acouchi (AF 21) killed on the 4th day of full vaginal opening, the ovaries were abnormally small; the right contained one small CL of ovulation and the left had only four accessory CL.

Accessory corpora lutea. Very few follicles were found in the early stages of luteinization but it would appear that some at least reached a diameter of about 800 μ before this process commenced (Pl. 1, Fig. 3). The fully formed accessories, however, rarely exceeded 0.5 mm in diameter (Pl. 1, Fig. 4). They were situated peripherally and, in their development, tended to displace the CL of pregnancy towards the centre which was composed chiefly of loose fibrous connective tissue and was highly vascular (see Pl. 2, Fig. 5).

The number of accessory corpora (see Table 3) increased during the first half of pregnancy and, thereafter, remained little changed. One animal (AF 1) was exceptional in that the available ovary consisted mainly of large cysts up to 2.5 mm in diameter which was not representative of the series. A large number of these corpora were also found in a non-pregnant acouchi (AF 16) 15 days after mating.

Luteal cells. The luteal cells in the true and accessory CL were uniform in appearance and indistinguishable. The cell boundary was visible mainly in those

EXPLANATION OF PLATE 2

FIG. 5. Ovary of acouchi (AF 15) on 60th day of pregnancy showing the peripheral disposition of accessory CL and displacement of CL of pregnancy towards the central core of connective tissue. $\times 15$.

FIG. 6. Portion of two adjacent CL of acouchi AF 1 on 75th day of pregnancy. *Top*—accessory CL with granulosa cells at periphery. *Bottom*—CL of pregnancy having lutein cells in contact with theca externa. $\times 400$.

FIG. 7. Peripheral portion of CL in AF 12 on 45th day of pregnancy showing the boundary of the luteal cells. $\times 250$.

FIG. 8. Part of centre of ovary of AF 20 on 95th day of pregnancy containing three degenerate eggs and the remains of atretic follicles. This region of the ovary in this pregnant acouchi is highly vascular. $\times 350$.

situated peripherally (Pl. 2, Fig. 7). These cells were rounded and had a moderately dense eosinophilic cytoplasm that was granular and frequently contained globules or vesicles. Their nuclei were proportionately large (about one-third the diameter of the cell), rounded and possessed one or more very prominent nucleoli.

The accessory CL frequently contained a narrow, peripheral layer of un-luteinized granulosa cells possessing densely basophilic nuclei which served as an additional distinguishing feature of these structures (Pl. 2, Fig. 6).

The mean diameter of the cells and their nuclei in the two types of CL is recorded in Table 4. In most of the animals of this small series, the cells in the true corpora were significantly larger than those in the accessories but their nuclei were of uniform size. Maximum cell diameter in the true corpora occurred in an acouchi (AF 6) examined on the 20th day of pregnancy but there is no evidence of a progressive decrease with the advancement of gestation. The luteal cells of the accessory CL in AF 6 were significantly larger than those in the animal (AF 16) examined 15 days after infertile mating. A significant difference was also noted in AF 1 between the size of the cells and their nuclei in the exceptionally large accessory CL and that of other accessory corpora; the latter corpora were comparable in size to those selected for cell measurements in all other animals of the series.

Follicles

Numerous non-antral and vesicular follicles were present in all ovaries examined histologically, except that of AF 1 which was cystic, but this specimen contained a follicle which was 1 mm in diameter and was one of the largest encountered. Several (usually about twelve) follicles varying between 500 and 700 μ , were present in the remainder and, although the theca interna was well developed (see Pl. 1, Fig. 2), none appeared to be undergoing pre-ovulatory changes such as are seen in the granulosa cells of the largest follicles of the domestic guinea-pig during pregnancy. Pycnosis of the granulosa cells was found in a large number of small Graafian follicles and their remains, particularly degenerate eggs, were frequently found in the connective tissue core of the ovary (Pl. 2, Fig. 8).

Progesterone assay

The results of progesterone assays of plasma and ovarian tissues are given in Table 5. In early pregnancy, the level of plasma progesterone was about 50 ng/ml and was maintained beyond mid-pregnancy, after which it decreased slowly. A rapid decrease in plasma progesterone concentration appeared to occur in the final stages of pregnancy or shortly after parturition. The plasma progesterone levels in non-pregnant animals were much lower than those of pregnant animals. Thus, in AF 13 which was examined on the 5th day *post partum* but was not suckling and had not ovulated, the plasma progesterone level was 7.5 ng/ml. Almost identical levels of progesterone of 12 ng/ml were found in two other non-pregnant animals that had ovulated; AF 21 (4th day of vaginal opening) and AF 16 (21st day of lactation and 15 days following infertile mating). Excluding the possible effects of lactation as insufficient data are

TABLE 4
THE LUTEAL CELLS OF TRUE AND ACCESSORY CORPORA LUTEA IN THE ACOUCHI

Acouchi no. (AF)	Condition at autopsy	CL of ovulation			Accessory CL		
		No. of CL	No. of cells measured	Mean diam. of cells ($\mu \pm S.E.$)	Mean diam. of nucleus ($\mu \pm S.E.$)	No. of cells measured	Mean diam. of cell ($\mu \pm S.E.$)
6	20 P	1	20	24.5 $\pm$ 0.70	9.3 $\pm$ 0.20	24	19.7 $\pm$ 0.55
17	29 P	0	—	—	—	28	16.2 $\pm$ 0.4
12	45 P	3	29	17.2 $\pm$ 0.36	7.4 $\pm$ 0.14	23	15.7 $\pm$ 0.28
15	60 P	1	30	19.0 $\pm$ 0.50	7.5 $\pm$ 0.10	12	19.7 $\pm$ 0.50
1	75 P	1	36	21.0 $\pm$ 0.30	7.6 $\pm$ 0.14	44*	21.0 $\pm$ 0.4
20	90 P	0	—	—	—	24†	19.3 $\pm$ 0.3
						34	19.0 $\pm$ 1.40
16	21 L; 15 p.c.	0	—	—	—	18	17.25 $\pm$ 0.34
14	10 L	0	—	—	—	25	20.0 $\pm$ 0.34
21	4 V						7.9 $\pm$ 0.12

* One very large accessory CL (diameter 0.75 mm).
† Accessory CL of normal size (diameter 0.50 mm).
Other abbreviations as in Table 1.

available, these figures probably suggest the pattern of plasma progesterone levels during the follicular and luteal phases of the oestrous cycle in the acouchi.

In pregnant animals, the greatest concentration of luteal progesterone (74 ng/ml) occurred during early gestation. Thereafter, it decreased slightly and was maintained at a level of about 40 ng/mg for the greater part of pregnancy. The high concentrations of luteal progesterone found in non-pregnant animals might have been due to a slower rate of hormonal release from this source.

Significant quantities of progesterone were found in the residual ovarian tissue which included the accessory CL. The concentrations are obviously not as important as the total amount of hormone given for this source in the interpretation of data, since the ovarian weight bears no relationship to the physiological state of the animal. The relative hormone contribution of residual tissue

TABLE 5
PROGESTERONE IN SYSTEMIC PLASMA, CORPORA LUTEA AND OVARIAN RESIDUES
INCLUDING ACCESSORY CORPORA LUTEA OF THE ACOUCHI

<i>Acouchi</i> no. (AF)	<i>Condition</i> at autopsy	<i>Plasma</i> (ng/ml)	<i>CL</i>		<i>Residual tissue</i>		<i>Contribution</i> of residual tissue (%)
			<i>Total</i> (ng)	<i>Concn</i> (ng/mg)	<i>Total</i> (ng)	<i>Concn</i> (ng/mg)	
6	20 P	47.8	740	74.0	254	6.7	25.4
17	29 P	54.9	911	45.6	227	17.9	20.0
12	45 P	51.4	649	37.5	458	12.9	41.5
15	60 P	50.5	142	34.6	272	4.0	65.6
1	75 P	34.7	768	47.1	236	8.2	23.5
20	90 P	23.6	—	—	728*	47.1	—
					728†	13.8	—
13	5 <i>p.p.</i>	7.5	—	—	105	2.1	—
16	21 L; 15 <i>p.c.</i>	12.1	1698	95.9	628	17.8	27.0
21	4 V	12.6	211.0	211.0	—	—	—

* Data for twenty-three small, dissected CL (total weight 10.2 mg); all were probably accessory CL but may have included one or a few very regressed CL of pregnancy.

† Data for the residue of the same ovary which still contained numerous accessory CL. Other abbreviations as in Table 1.

was estimated by assuming equal rates of release from true and accessory CL calculated as a proportion of the total. Table 5 reveals that the contribution from this source increased during the latter half of gestation as if to supplement the function of the true CL. Proof of the hormonal activity of the accessory CL in late pregnancy is provided by the data given for AF 20, in which the CL of pregnancy had regressed to a size that rendered them indistinguishable from the accessories. In this animal, the latter CL probably formed the only source of luteal progesterone.

DISCUSSION

As only a limited number of animals was available for these investigations, it seemed justifiable at the outset to combine a histological examination on one ovary with a biochemical study on the second ovary of every animal. The ovary containing the more obvious CL was used for the latter study, and it soon

became apparent that the histological observations would be limited by the absence of true CL in some of the animals. Very little difficulty was encountered in the identification of the two types of CL except in one acouchi (AF 12), in which some doubt existed about three corpora of intermediate size; they were regarded on other histological evidence as having resulted from ovulation (see Table 3).

The growth of the true CL of the acouchi is completed about the end of the 2nd week of the 14-week gestation period, and is comparable with that of the guinea-pig in which growth is complete towards the end of the 3rd week of a 10-week gestation period (Rowlands, 1956). The pattern of luteal growth in the pregnant coypu is very different (Rowlands & Heap, 1966). In this species, growth of the CL was almost imperceptible for the first 6 weeks and maximum size was not attained until about the 13th week of the 19-week gestation.

In the chinchilla, which is the only other hystricomorph rodent for which comparable data are available, Weir (1967) has reported the presence of a large number of CL of variable size and histological appearance which made identification of the 'original' CL most difficult. However, a few large CL which gradually increased in size were found between the 3rd and 13th week of pregnancy; thereafter, regression occurred until parturition 3 weeks later. The pattern of luteal growth in the chinchilla would seem, therefore, to be more closely allied to that of the guinea-pig and the acouchi than to that of the coypu.

Accessory CL are found during the cycle and pregnancy in several species allied to the acouchi (see Mossman, 1966; Weir, 1967), but occur very infrequently in the domestic guinea-pig. As a general rule, these structures are smaller than the CL of ovulation and are very numerous as, for example, in the Canadian porcupine (Mossman & Judas, 1949), chinchilla (Weir, 1967) and in the acouchi. In the mountain viscacha (*Lagidium peruanum*), however, only a limited number (ten to twelve) are produced and they usually grow to about one-half, and occasionally may equal, the size of a single CL of ovulation (Pearson, 1949). In view of the existence of accessory CL in several hystricomorph rodents, the total amount of lutein tissue in the ovary should be taken into account when considering the progesterone requirements of pregnancy.

Most observers have commented on the histological similarity of the luteal cells of the true and accessory CL in hystricomorphs; and the present study has shown that the acouchi is not exceptional; only slight differences were found between the mean diameter of the luteal cells in both types of corpora. Cell and nuclear diameters were greatest (24.5 and 9.3 μ respectively) in a true CL in early pregnancy (see AF 6) which probably coincided with the completion of its growth. A similar relationship, but at a much later stage of pregnancy (12 to 14 weeks), existed in the coypu (Rowlands & Heap, 1966); at this time the mean diameter of the luteal cells had reached 34.5 μ .

The levels of progesterone in the plasma of non-pregnant and pregnant acouchis are compared (Text-fig. 1) with those in the guinea-pig (Heap, Perry & Rowlands, 1967) and the coypu (Rowlands & Heap, 1966). In non-pregnant animals, the levels are uniformly low (2 to 12 ng/mg) in all three species but specific differences appear to exist during pregnancy in the maximum levels reached and in their duration. This level is attained early in the pregnant

guinea-pig and acouchi but not until the latter half of gestation in the coypu. Preliminary studies on plasma progesterone levels in the chinchilla have indicated a rise to a maximum of about 45 ng/ml at about the 13th week of pregnancy followed by a very rapid decrease near to term (Weir, 1967). The plasma progesterone level of all these species follows closely the pattern of growth and decline of the true corpus luteum.

On the other hand, progesterone levels in the CL of ovulation of non-pregnant acouchis are much higher (see Table 5) than those reported in the guinea-pig (16 ng/mg; Rowlands & Short, 1959; Heap *et al.*, 1967). These levels are much lower in pregnant than in non-pregnant acouchis, which suggests that the rate

TEXT-FIG. 1. Comparison of plasma progesterone levels in non-pregnant, pregnant and *post-partum* guinea-pigs (■), acouchi (●) and coypu (▲). In the case of pregnant animals, the stage at which blood plasma was sampled is expressed on the abscissa as the percentage of the gestation period (■ = 68 days; ● = 100 days; ▲ = 132 days).

of hormone-release is probably much greater in order to meet the demands of gestation. They are, however, considerably higher than those found in the coypu (27 ng/mg) and the guinea-pig (31 ng/mg).

The contribution of the accessory CL to the level of ovarian progesterone is considerable at all stages of gestation and rises to more than 50% at mid-pregnancy. In one near-term animal (AF 20) in which no distinguishable corpus luteum of ovulation was found, the accessory CL probably provided all the available progesterone, other than that which might be secreted by the placenta. The ovarian residues of the guinea-pig, in which there are no accessory CL, do not contain measureable quantities of progesterone.

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