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Plasma Protein Distribution and Turnover in the Female Baboon

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The menstrual cycle of the baboon is accompanied by periodic variations in the concentration and total mass of circulating protein. The available evidence indicates that these unusual fluctuations are related to the hypertrophy and involution of perineal tissue which occur in step with the menstrual cycle in this species. Thus the mass of circulating protein decreases during perineal growth, which accompanies the follicular phase of the cycle; with the onset of the luteal phase the perineum undergoes rapid involution and, at the same time, the

plasma protein mass increases considerably (Cohen, 1955).

The present study employing ¹²⁵I-labelled plasma protein was undertaken in order to obtain more precise information about variations in plasma protein distribution and turnover in the adult female baboon. The validity of metabolic data obtained through the use of iodinated protein has been seriously questioned in recent years (Berson, Yalow, Schreiber & Post, 1953; Armstrong *et al.* 1955; Masouredis & Beckmans, 1955; Volwiler *et al.* 1955).

However, investigations conducted in this Laboratory have shown that plasma protein iodinated according to the method described by McFarlane (1956) provides data concerning the distribution and elimination of albumin and globulin closely similar to that obtained with the corresponding ^{14}C -labelled fractions in both the rabbit (Cohen, Holloway, Matthews & McFarlane, 1956) and the rat (unpublished observations).

The use of iodinated protein is advantageous since it permits labelling of the animal's own plasma. Moreover, since ^{131}I liberated by catabolism is promptly excreted (Dixon, Bukantz & Dammin, 1951; Cohen *et al.* 1956) the rate of plasma protein catabolism and the pool size can be estimated repeatedly during the course of observation. These measurements have proved particularly useful in the female baboon, in which rates of plasma protein synthesis and catabolism and the size of the exchangeable protein pool alter in step with the menstrual cycle.

METHODS

Animals

This investigation was conducted on two mature female Chacma baboons (*Papio ursinus*) known to be at least 8 years of age. The premenstrual weights of these animals were 17.1 and 21.6 kg. respectively. During experiments the baboons were separately housed in metabolism cages. Two days before the injection of iodinated protein, and throughout the subsequent period of observation, NaI (100 mg./l.) was added to the drinking water. The animals received 100 g. of pelleted diet no. 18 (Bruce & Parkes, 1946) and 1.5 kg. of either potatoes or carrots daily.

Urine was collected at 10 a.m. each morning. The grid and funnel of the cage were washed down with 500 ml. of approximately 0.5 N-NaOH, and this was added to the urine.

Alterations in the size of the perineal swelling characteristic of the adult female baboon were plotted by daily measurement of the distance between ischial callosities, and on this basis the menstrual cycle was divided into two parts (Gillman & Gilbert, 1946). The first, referred to as the follicular phase, or period of turgescence, commences on the first day of menstruation and is characterized by progressive enlargement of the perineal swelling. The subsequent luteal phase is associated with involution of perineal tissue and is terminated by the onset of the next menstrual period.

Preparation of material for iodination

In each experiment the animal's own plasma protein was used for iodination. Whole plasma was obtained by collecting 6 ml. of blood in 1 ml. of an oxalate solution (1.03% sodium oxalate and 1.09% ammonium oxalate, w/v). Albumin was prepared by fractionation of 300 mg. of baboon serum protein by zone electrophoresis according to the method described by Flodin & Porath (1954) and Porath (1954). The column, which consisted of acetylated cellulose (Campbell & Stone, 1956), was 40 cm. long and 2.5 cm. in diameter, and was surrounded by a jacket through which water at 2° was circulated. A borate buffer (0.0115 M- $\text{Na}_2\text{B}_4\text{O}_7$ and 0.0155 M- NaH_2PO_4 , pH 8.6) was used. The electrophoresis was

carried out at 22 mA, which required about 450 v. Cooling of the column was maintained during collection of 3 ml. volumes of eluate (12 ml./hr.) on a fraction collector. The albumin peak was located by measurement of ultraviolet absorption at 280 m μ . The solution containing albumin was pooled and reduced to a volume of 3 ml. by pressure dialysis (10 lb./sq.in.) against 0.9% NaCl at 4°. A specimen of albumin (90 mg.) containing 4% of α_1 - and α_2 -globulins as estimated by paper electrophoresis was obtained by this procedure.

Method of iodination

Plasma protein labelled with ^{131}I was prepared according to the procedure described by McFarlane (1956). A volume (3 ml.) of plasma (150–250 mg. of protein) or albumin solution (90 mg. of protein) was mixed immediately before iodination with 1.5 ml. of glycine buffer [0.6 g. of glycine dissolved in 8 ml. of 5.85% NaCl (w/v) and 2 ml. of N-NaOH, pH 9.3]. The required amount of carrier-free ^{131}I -iodide was added to 0.1 ml. of a solution containing 0.40 mg. of NaI and 0.15 mg. of NaIO_3 . Iodine was liberated from this solution by the addition of one drop of N-HCl immediately before jet mixing with protein. The iodinated protein solution was transferred to an Amberlite IR4B column, previously buffered with histidine at pH 6.5 and saturated with NaCl. The final iodinated protein solution was collected from the column in a volume of approximately 6 ml.; less than 1.5% of the total radioactivity was present in the supernatant after precipitation of protein with 10% of trichloroacetic acid (TCA) at 20°. There was 15–20% of the initial radioactivity used bound to protein. When whole plasma was iodinated the relative specific activities of fractions separated by zone electrophoresis were albumin 100, α_1 -globulin 23, α_2 -globulin 20, β -globulin 30, γ -globulin 35.

Injection of labelled protein

Between 0.08 and 0.15 g. of labelled plasma protein in 5 ml. was injected into the antecubital vein. The washings from syringe, needle and vessel, which had contained exactly 5 ml. of the solution, were collected and suitably diluted for counting. The proportion of the measured volume which had not been injected was thus ascertained, and the dose administered determined by difference.

Blood samples

Five minutes after injection 3–5 ml. of blood was withdrawn from the opposite antecubital vein into 0.05 ml. of heparin solution (1000 i.u./ml.). Similar blood samples were collected at intervals of 1–3 days throughout the period of observation.

Preparation of protein samples for counting

Total plasma protein. Samples of plasma (0.1–0.5 ml.) were diluted to 3 ml. with 0.9% NaCl (w/v) for ^{131}I counting.

Salt fractionation: Fibrinogen. Samples of plasma (1–3 ml.) were diluted to 5 ml. with 0.9% NaCl. Fibrinogen was twice precipitated by addition of 27% (w/v) aqueous Na_2SO_4 solution to give a concentration of 10%. The second precipitate was dissolved in about 15 ml. of 0.9% NaCl. Approximately 0.05 ml. of 0.5% (w/v) protamine sulphate and 0.05 ml. of 0.5% (w/v) thrombin were added and clotting was induced by incubation at 37° for 0.5 hr. The fibrin clot was collected on a glass rod and washed in 0.9% NaCl and then in water. Finally, the fibrin was dissolved

in 1 ml. of N -NaOH and made up to 3 ml. with water for counting. Globulin was precipitated from the supernatant at 10% of Na_2SO_4 by the addition of 27% Na_2SO_4 to give a final concentration of 18% of Na_2SO_4 . The precipitate was dissolved in 5 ml. of 0.9% NaCl and reprecipitated in 18% Na_2SO_4 . This second precipitate was dissolved in 3 ml. of 0.9% NaCl for counting. Albumin present in 3 ml. of the supernatant at 18% of Na_2SO_4 was used for ^{131}I counting. Electrophoretic analysis revealed that globulin fractionated by the method given above contained predominantly β - and γ -globulins, and α -globulin and albumin comprised about 10% of the total. The albumin fraction contained 25–30% of α_1 - and α_2 -globulins.

Zone electrophoresis. Samples of serum obtained after injection of labelled albumin were fractionated by electrophoresis on a cellulose column under the conditions described above for the preparation of albumin. During electrophoresis of these samples the column was cooled with circulating tap water. The ultraviolet absorption of 3 ml. volumes of eluate collected on the fraction cutter were determined at 280 $m\mu$. Four tubes containing the albumin were pooled and protein was precipitated at a concentration of 10% of TCA at 80°. This precipitate was centrifuged down, dissolved in 1 ml. of N -NaOH and finally made up to 3 ml. with water for counting.

Analytical methods

Protein estimations. These were done on measured fractions of solutions used for ^{131}I counting, according to the biuret method of Gornall, Bardawill & David (1949).

Measurement of radioactivity. Solutions were measured by scintillation counting, with a NaI crystal in conjunction with an 11-stage electron multiplier. The crystal had a central well to hold 3 ml. in a glass tube, and measured γ -radiation with an efficiency of 33%. The maximum error of counts recorded in this study is $\pm 3\%$.

Moving-boundary electrophoresis. Electrophoresis was carried out in a Tiselius apparatus with a buffer containing 0.15M-NaCl and 0.05M sodium barbitone adjusted to pH 8.6 by addition of N -HCl (7.4 ml./l.). The proportion of each component was determined from the descending pattern by constructing symmetrical curves for each peak (Svedberg & Pedersen, 1940).

Paper electrophoresis. Paper electrophoresis was performed on hanging strips of Whatman filter paper no. 1 according to the method described by Flynn & de Mayo (1951). A veronal buffer (0.05M, pH 8.6) was used. The strips were stained with azocarmine without previous drying. An EEL scanner (Evans Electroselenium Ltd., Harlow, Essex) with a green filter was employed for the photometry of stained paper strips.

Ultracentrifugal analysis. Protein solutions were dialysed against a buffer containing 0.01M- Na_2HPO_4 , 0.04M- KH_2PO_4 and 0.40M-NaCl (pH 5.9) and examined in a Spinco analytical centrifuge.

Analysis of perineal tissue. At intervals during the course of two experiments the volume of the perineal swelling was determined in the anaesthetized baboon by means of a plaster cast, as previously described (Cohen, 1955). An injection (3.0 ml.) of a 0.3% (w/v) solution of Evans Blue (T1824 British Drug Houses Ltd., Poole) was then made intravenously. Five minutes later a blood sample was taken and a wedge of tissue 2–3 in. in depth and weighing 3–5 g.

was excised from the perineal swelling approximately halfway between the mid-line and the lateral margin. The skin of this biopsy sample was removed, and the underlying gelatinous connective tissue used for analysis.

A portion of the biopsy sample was cut into segments of approximately 2–4 mm.², and within 10 min. of removal was centrifuged (1500 rev./min. at 2°) for about 0.5 hr. By this means a straw-coloured fluid comprising about 40% of the volume of the sample was obtained. The plasma content of this fluid was determined by comparing the concentration of Evans Blue in the sample with that in plasma withdrawn at the time of biopsy. Evans Blue was estimated by light-absorption at 620 $m\mu$. Since the perineal fluid always contained less than 0.5% of plasma, the radioactivity and protein content of a sample of perineal fluid were regarded as providing a measure of the specific activity ($\mu C/mg.$) of perineal extravascular plasma protein (P).

The volume of a portion of the biopsy specimen was measured after centrifuging in a graduated tube. This sample was dissolved in 0.1N-NaOH and its protein content and radioactivity were determined. The blood content of another tissue slice of known volume was estimated by the method of Gordon & Nurnberger (1955). These determinations, in conjunction with the measurement of perineal volume, provided estimates of total protein (A), total radioactivity (B) and total blood content (C) of the perineal connective tissue. The radioactivity of blood ($\mu C/ml.$) withdrawn at the time of biopsy was measured (D). The total quantity of plasma protein present in the extravascular tissue of the perineum (E) was calculated from

$$E = (B - C \times D)/P.$$

The non-plasma protein of the perineum was equal to ($A - E$). The mass of plasma protein present in tissues other than the perineum (J) was calculated from

$$J = [F - (G + B)]/H,$$

where F is the dose of ^{131}I administered, G is the cumulative excretion of ^{131}I , and H is the specific activity of plasma protein at the time of biopsy.

On the 11th day after injection of ^{131}I -labelled albumin a sample of perineal fluid was fractionated by zone electrophoresis and the specific activity of the albumin fraction determined. The total quantity of extravascular albumin in the perineum was calculated as above.

Calculation of catabolic rate and pool size. Three daily samples of faeces collected at intervals after the injection of iodinated plasma were found to contain 1.4–3.0% of the ^{131}I present in the corresponding 24 hr. sample of urine. The daily excretion of ^{131}I is given by

$$24 \text{ hr. urinary } ^{131}I + 3\%.$$

The ^{131}I retained in the body is given by

$$\text{dose of } ^{131}I - (\text{cumulative excretion of } ^{131}I + ^{131}I \text{ removed in blood samples}).$$

The percentage of the pool catabolized/24 hr. is given by

$$100 \times \text{daily } ^{131}I \text{ excretion} / ^{131}I \text{ retained.}$$

The exchangeable protein pool (g.) is given by

$$^{131}I \text{ retained } (\mu C) / \text{plasma protein specific activity } (\mu C/g.).$$

Table 1. *Elimination of ^{131}I from plasma and urine during the initial 100 hr. after injection of iodinated plasma protein*

Baboon no.	Wt.* (kg.)	Perineal measurement† (in.)	Dose of ^{131}I (μC)	(1) Plasma protein specific activity at 100 hr. (%)	(2) Urinary excretion of ^{131}I at 100 hr. (%)	(3) Plasma protein pool (g.)
321	17.1	11	558	33.5	25.4	130
		15	416	18.0	22.8	154
		15	425	17.5	24.6	150
351	21.6	11½	195	32.5	22.5	128
		15	425	17.5	24.6	150
		15	425	17.5	24.6	150

* Weight during perineal resorption.

† Perineal measurement on the day of injection.

(1) Plasma protein specific activity at 100 hr. $\times 100$.

(2) Plasma protein specific activity at 5 min. $\times 100$.

(3) Total ^{131}I excreted in urine at 100 hr. $\times 100$.

Dose of ^{131}I administered

(3) Calculated from the dose of ^{131}I administered divided by the zero time extrapolation value of the slow decay curve of plasma specific activity.

RESULTS

Results obtained with ^{131}I -labelled plasma protein

Effect of perineal size on the initial elimination of ^{131}I from plasma and urine. ^{131}I -Labelled plasma protein was administered to both baboons during the phase of perineal involution; 3–9 months later each received a second dose of labelled protein on the 8th–9th day of perineal turgescence (Table 1).

The first blood sample was taken 5 min. after injection. In each experiment the specific activity of plasma protein ($\mu\text{C}/\text{mg.}$ of protein) showed an initial rapid fall followed by a slower rate of decay. In both animals the decline of specific activity of plasma protein over the first 4 days was more rapid during perineal turgescence than during the luteal phase of the cycle (Fig. 1). Thus the proportion of labelled protein retained in the circulation 100 hr. after injection was 33 % during perineal resorption and only 18 % during turgescence (Table 1). The urinary excretion of ^{131}I , on the other hand, was constant during both phases of the cycle (22.4–25.4 % of the dose after 100 hr.).

These facts indicate that the rapid initial elimination of ^{131}I from plasma during the follicular phase results from dilution of labelled molecules by an enlarged protein pool. From the dose administered and the extrapolation to zero time of the curve of the slow decay of the plasma protein the size of the exchangeable protein pool was estimated to be 128 and 130 g. during perineal resorption and 150 and 154 g. on the 8th–9th day of perineal hypertrophy.

Variations in the plasma and urinary elimination of ^{131}I during the course of the menstrual cycle. The plasma and urinary elimination of ^{131}I were followed in two baboons for a period of 7 weeks after injection of iodinated protein. One of these animals received 558 μC of ^{131}I -labelled plasma 4 days before

Fig. 1. Elimination of ^{131}I from plasma and urine after injection of iodinated plasma protein. Baboon 321: ■, 558 μC injected during complete perineal involution; ●, 416 μC injected on seventh day of perineal turgescence. Baboon 351: ○, 195 μC injected during complete perineal involution; □, 425 μC injected on ninth day of perineal turgescence. Plasma protein specific activity expressed as a percentage of the value at 5 min. after injection.

the onset of menstrual bleeding (Fig. 2). During the first 3 days the usual rapid decline in the specific activity of the plasma protein was observed. This was followed during the remainder of the follicular phase by a slower, approximately constant, exponential rate of decay. Globulin ($t_{1/2}$, 12 days) was eliminated more rapidly than albumin ($t_{1/2}$, 16 days), and fibrinogen far more rapidly than either ($t_{1/2}$, 3 days). The rate of plasma protein synthesis calculated from the apparent half-life of total plasma protein [$0.693/t_{1/2}$ (days) $\times 100$] was 5.1 % of the pool/day. The rate of catabolism estimated from

24-hourly urinary excretion of ^{131}I was 1.9–4.3 % of the pool/day. The total pool size increased progressively from 117 g. at the onset of menstrual bleeding to 192 g. at the end of perineal turgescence.

With the onset of perineal involution the specific activity of the plasma protein rose slightly and then diminished for a period of 4 days. Between the fifth and twelfth days of the luteal phase the decay of plasma radioactivity ceased and the specific activity of total protein varied by less than 1 % in five successive estimations made during this time. The decay curves of albumin and globulin did not follow identical patterns, but in general elimination rates of both were considerably slower than those observed during perineal turgescence. These results indicate that the rate of synthesis of plasma protein is considerably reduced or even completely inhibited during the latter part of perineal involution. The

rate of catabolism calculated from the excretion of iodide (2.6–3.4 % of the pool/24 hr.) rose above the level observed during the latter 2 weeks of turgescence. Between the fifth and twelfth days of the luteal phase the pool size diminished progressively from 195 to 154 g. It will be observed from Fig. 2 that the pool size at the beginning of the experiment was 117 g.; at the end of the experiment, when a comparable stage of perineal involution had been reached the apparent pool size was 154 g. The significance of this discrepancy is discussed below.

In a second experiment the other baboon received 425 μC of ^{131}I -labelled plasma protein on the ninth day of perineal turgescence. The elimination of isotope from plasma and urine was followed during the remainder of that menstrual cycle and the initial 18 days of the next (Fig. 3). The results were similar to those described above (Table 2). The specific activity of the plasma protein again decayed exponentially during perineal hypertrophy. The estimated rate of synthesis exceeded that of catabolism and the pool size increased by 43.5 g. and 55.5 g. during the first and second follicular phases

Fig. 2. Plasma protein turnover during the baboon menstrual cycle. ^{131}I -Labelled plasma (558 μC) was injected 4 days before the onset of menstrual bleeding, $\square$. For method of making perineal measurement and for calculation of pool size and rate of plasma protein catabolism, see text.

Fig. 3. Plasma protein turnover during two consecutive menstrual cycles in the baboon. ^{131}I -Labelled plasma (425 μC) was injected on the ninth day of the cycle. Menstrual bleeding, $\square$. For method of making perineal measurement and for calculation of pool size and rate of plasma protein catabolism, see text.

Table 2. *Turnover rates of total plasma protein and albumin alone during the phases of perineal hypertrophy and involution in the female baboon*

See text for methods of calculating rates of synthesis and catabolism and plasma protein pool size.

Fig. no.	¹³¹ I-labelled protein	Perineal hypertrophy			Perineal involution		
		Synthesis (% of pool/24 hr.)	Catabolism (% of pool/24 hr.)	Alteration of pool size (g.)	Synthesis (% of pool/24 hr.)	Catabolism (% of pool/24 hr.)	Alteration of pool size (g.)
2	Whole plasma	5.0	1.9-4.3	+75	0-5.5	2.6-3.4	-41
3	Whole plasma	5.5*	1.7-4.0*	+55.5*	1.4-6.0	3.8-6.3	-44.5
8	Albumin	6.4	3.3-6.0	+11	2.6-4.0	2.4-14.4	-13

* Calculated from results obtained during second turgescient phase of experiment shown in Fig. 3.

Fig. 4. Sedimentation boundary diagrams of plasma and perineal fluid proteins. Samples obtained on seventeenth day of perineal turgescence. Protein concentration of samples (g./100 ml.): plasma, 1.20; perineal fluid, 1.25. Measurements were made at 996 rev./sec. and at approximately 20°. Time after full rotor speed, 128 min.

respectively. The rate of catabolism estimated from the urinary excretion of ¹³¹I increased during perineal involution, and the rate of decay of the specific activity of total protein diminished considerably between the ninth and thirteenth days of the luteal phase; during this time the pool size decreased from 201 to 156 g. A discrepancy in pool-

Fig. 5. Electrophoretic diagrams of ascending and descending boundaries of plasma and perineal fluid. Tiselius apparatus. Protein concentration (g./100 ml.): plasma, 1.47; perineal fluid, 1.22. Plasma, 360 min., perineal fluid, 430 min., after the beginning of migration. Samples obtained on seventeenth day of perineal turgescence. For details of buffer, see text.

size estimation similar to that referred to in the previous experiment was again evident. Thus during the pre-menstrual phase of complete perineal resorption, which occurred after 3-4 weeks of observation, the pool size varied between 156 and 162 g., whereas the initial pool size in the same baboon previously injected during perineal involution was only 128 g. (Table 1).

Analysis of perineal protein. Information about the relationship between plasma and perineal proteins has been obtained by analysis of three successive samples of perineal tissue excised from a baboon which received 416 μ c of ¹³¹I-labelled plasma protein on the seventh day of the cycle. Centrifuging perineal tissue according to the method described above yielded a straw-coloured, somewhat viscous fluid comprising 30-40 % of the volume of the tissue and containing less than 0.5 % of plasma as determined by the content of Evans blue injected 5 min. before biopsy. The protein concentration of three samples of perineal fluid obtained at intervals during turgescence was 20-23 mg./ml., and the corresponding plasma samples contained 52-53 mg. of protein/ml. The same electrophoretic and ultracentrifugal components were present in perineal fluid and plasma (Figs. 4, 5); however, the

Table 3. *Electrophoretic and ultracentrifugal analyses of perineal fluid and plasma*

Samples analysed by electrophoresis and ultracentrifuging were withdrawn on the seventeenth day of perineal turgescence.

	Protein* concn. (mg./ml.)	Electrophoretic analysis % (and mobility†)				
		Globulins				Albumin
		α_1^-	α_2^-	β^-	γ^-	
Plasma protein	52-53	33.9 (-6.0)	8.5 (-4.8)	19.9 (-3.9)	17.0 (-3.3)	20.7 (-1.2)
Perineal fluid protein	20-23	42.3 (-5.8)	5.7 (-4.9)	17.5 (-4.0)	17.0 (-3.1)	17.5 (-1.2)
Ultracentrifugal analysis						
		$S_{20}^\ddagger$	% of total	$S_{20}^\ddagger$	% of total	
Plasma protein		3.77	80.9	5.69	19.1	
Perineal fluid protein		3.83	87.8	5.85	12.2	

* Range in three successive samples.

† 10^5 cm.²/v/sec.

‡ S_{20} values corrected to water at 20°. Partial specific volume of protein taken as 0.749. Protein concentration of samples analysed by ultracentrifuging: plasma, 1.20%; perineal fluid, 1.25%.

proportion of albumin and of the more slowly sedimenting ultracentrifugal component was somewhat lower in plasma (Table 3).

Specific activity of plasma and perineal fluid protein. The first biopsy of perineal tissue was taken 5 min. after injection of the labelled plasma. At this time the specific activity of the protein of perineal fluid was less than 1% of that of the plasma protein; this degree of labelling was attributed to the plasma present in the perineal fluid sample. Four days later the specific activity of the protein of perineal fluid had increased above that of plasma (Fig. 6). On the tenth day the specific activity of the perineal fluid was 1930 and that of plasma 1340 $\mu\text{C}/\text{mg.}$ of protein. Specific activities of both albumin and globulin (separated by Na_2SO_4 fractionation) were greater in perineal fluid than in plasma. However, fractionation by zone electrophoresis revealed that the specific activity of albumin was closely similar in both fluids on the tenth day of the experiment, and the specific activities of all globulin fractions were higher in perineal fluid than in plasma (Fig. 7).

Protein content of perineal swelling. The relative specific activities of plasma and perineal fluid proteins and their electrophoretic and ultracentrifugal identity indicate that all the protein present in perineal fluid is derived from the plasma. It is possible by analysis of perineal tissue to estimate the total extravascular plasma protein content of perineal connective tissue (see Methods). In the baboon subjected to repeated biopsy the volume of the perineum increased from 1590 to 3080 ml. and its total protein content rose from 53 to 101 g. between the seventh and seventeenth days of the follicular phase (Table 4). On the eleventh day the perineum contained 74 g. of total protein, of which 36 g. was extravascular plasma protein. Six days later an additional 27 g. of protein had accumulated in the

Fig. 6. Protein specific activities of plasma (continuous lines) and perineal fluid (broken lines). ^{125}I -Labelled plasma protein ($416\mu\text{C}$) was injected on seventh day of perineal turgescence. Albumin and globulin were fractionated by Na_2SO_4 (see text). Samples of perineal fluid were obtained 5 min., 96 hr. and 240 hr. after administration of the labelled protein. The protein of extravascular perineal fluid was unlabelled at 5 min.

Fig. 7. Specific activities of plasma and perineal fluid proteins. ^{131}I -Labelled plasma protein ($416\mu\text{C}$) was injected on seventh day of cycle. Samples withdrawn on seventeenth day of cycle and fractionated by zone electrophoresis (see text). Fractions used for specific activity determinations were concentrated by pressure dialysis, and analysed by paper electrophoresis.

perineum, and 15 g. of this consisted of extra-vascular plasma protein. At the same time the amount of plasma protein present in tissues other than the perineum diminished by 10 g., so that the total pool increased by 15 g. It is apparent from Fig. 6 that from the fourth day of the experiment the average specific activity of protein in the total pool is higher than that in plasma alone. Estimates of pool size based on the specific activity of plasma samples are therefore erroneously high (Table 4).

Results obtained with ^{131}I -labelled albumin

^{131}I -Labelled albumin ($522\mu\text{C}$) prepared by zone electrophoresis was administered to one of the baboons on the sixth day of turgescence and observations were continued throughout the menstrual cycle (Fig. 8). From the second day after injection and for the remainder of the follicular phase the specific activity of plasma albumin declined at an approximately constant exponential rate corresponding to 6.4% of the exchangeable albumin pool/24 hr. The rate of catabolism calculated from ^{131}I excretion was 3.3–6.0% of the pool/24 hr. The rate of albumin synthesis consistently exceeded

that of catabolism, and the pool size increased from 53 g. on the eighth day to 64 g. on the fifteenth day of the cycle. On the eleventh day after injection of the isotope, analysis of a sample of perineal tissue revealed that the specific activity of perineal fluid albumin corresponded exactly with that of plasma albumin. The volume of the sexual swelling at the time of biopsy was 3750 ml.; the size of the total exchangeable albumin pool was 62 g., and 39 g. of albumin was present in the extravascular tissue of the perineal swelling. The biopsy was followed by an increased rate of ^{131}I excretion. This postoperative catabolic effect appears to be of brief duration since, in another baboon which received iodinated plasma, the urinary excretion of ^{131}I returned to the pre-operative level on the third day after biopsy (Fig. 9).

With the onset of perineal involution the rate of decline of the specific activity of plasma albumin diminished considerably (4.0% for 7 days and then 2.6% of the albumin pool/24 hr.). The rate of catabolism estimated from ^{131}I excretion rose to 14.4% of the albumin pool/24 hr. on the first day of the luteal phase, declined rapidly to 3% by the seventh

Table 4. *Volume and protein content of the perineal swelling during the turgescence phase of the cycle*

For methods of estimating protein content and total radioactivity see text. In the last two columns:

(1) addition of perineal plasma protein and extraperineal plasma protein;

(2) $\frac{\mu\text{C in body at time } t}{\text{plasma specific activity at time } t} (\mu\text{C/g.}) \cdot$

Days after injection	Day of cycle	Perineal vol. (ml.)	$\mu\text{C of }^{131}\text{I}$ retained			Specific activities ($\mu\text{C/mg.}$)			Protein content (g.)			Estimate of plasma protein pool (g.)	
			Total	Perineum	Extra-perineal	Plasma protein	Perineal fluid protein	Perineal plasma protein	Residual perineal protein	Extra-perineal plasma protein	Perineal plasma protein	By addition (1)	By dilution (2)
0	7	1590	416	—	—	—	—	—	—	—	—	—	—
4	11	2460	327	102	225	2420	2860	36	38	93	61	129	135
10	17	3080	227	117	110	1340	1930	61	40	83	83	144	170

Fig. 8. Albumin turnover during the baboon menstrual cycle. ^{131}I -Labelled albumin ($522\mu\text{C}$) was injected on the sixth day of perineal turgescence. Menstrual bleeding, $\square$. On the eleventh day of the cycle a biopsy of perineal tissue was taken. Albumin was prepared and subsequently isolated from plasma and perineal fluid by zone electrophoresis. For calculation of pool size and rates of synthesis and catabolism, see text.

day and remained between 2 and 3 % during the remainder of the cycle. The albumin pool diminished from 57 to 44 g. during the initial 3 days of perineal involution and thereafter showed little variation (Fig. 8).

DISCUSSION

The perineal swelling of the female baboon consists essentially of connective tissue. During turgescence there occurs a dispersion of cells indicative of a considerable accumulation of intercellular ground substance (Zuckerman & Parkes, 1932). A slightly viscous fluid with a protein content of about 20 mg./ml. and comprising 30–40 % of the volume of the sample is obtained from perineal tissue by light centrifuging in the cold. This fluid contains less than 0.5 % of plasma and is presumably expressed mainly

Fig. 9. Effect of perineal biopsy upon rate of plasma protein catabolism. ^{131}I -labelled plasma protein was injected on seventh day of perineal tumescence. Perineal biopsy was performed on the fourth day of the experiment. For method of calculating rate of catabolism, see text.

from the ground substance of perineal connective tissue. The electrophoretic and ultracentrifugal components of perineal fluid are similar to those of plasma. The ultracentrifugal fraction, thought to be a mucoprotein which has been observed in fluid expressed from the swollen sex skin of the rhesus monkey (Ogston, Philpot & Zuckerman, 1939), was not evident in perineal fluid examined under the conditions described above. These observations in the baboon suggest that perineal fluid proteins are derived from plasma protein; this view is confirmed by comparison of the specific activities in plasma and perineal fluid proteins after the intravenous injection of ^{131}I -labelled protein (Figs. 6, 7).

During the premenstrual phase of the cycle the resorbed perineal swelling contains 20–30 g. of protein; approximately 50 g. of additional protein is deposited in perineal connective tissue during tumescence (Cohen, 1955). In the present tracer experiments the mass of extravascular perineal plasma protein has been estimated on the basis of the total ^{131}I content of the swelling, the assumption being that the specific activity of plasma protein throughout the swelling is the same as that of perineal fluid protein. These estimations indicate that about 4 g. of protein accumulates in the tumescent perineum/day, and this increment consists almost exclusively of extravascular plasma protein which may or may not be partly intracellular (Table 4).

Comparison of the specific activities of plasma and perineal fluid proteins after injection of ^{131}I -labelled plasma indicates that perineal protein derived from plasma continues to exchange with circulating protein during growth of the sexual

swelling (Fig. 6). The exchange of albumin is sufficiently rapid to maintain equilibrium, as shown in two experiments in which the specific activities of plasma and perineal fluid albumin separated by electrophoresis were identical 10 and 11 days after injection of the isotope (Figs. 7, 8). The specific activities of perineal globulin fractions, on the other hand, were higher than those of plasma on the tenth day after injection of labelled plasma (Fig. 7). This suggests that plasma and perineal globulins exchange relatively slowly; with the progress of the experiment the specific activity of perineal globulin at any time is equivalent to that of plasma globulin some days previously.

Hypertrophy of the perineum is associated with a decrease in the mass of circulating protein (Cohen, 1955). The present tracer experiments indicate that at the same time the total plasma protein pool increases considerably in size. Thus the association of a tumescent perineum with a relatively rapid initial elimination of ^{131}I from the circulation presumably results from dilution of labelled molecules by an enlarged protein pool, since the initial urinary elimination of the isotope does not alter with the state of the perineal swelling (Fig. 1). However, the extent to which the total plasma protein pool alters in size during the course of the menstrual cycle has not been accurately determined. Pool-size values calculated by extrapolation of the curve of slow plasma decay to zero time (Table 1) are based on the doubtful assumption that throughout the process of equilibration a constant proportion of the labelled molecules retained in the body is degraded per unit time (Cohen *et al.* 1956). Measurements based upon the ^{131}I content of the body and the specific activity of the plasma protein at the same time provide unduly high values (Table 4) because, with the progress of the experiment, the specific activity of extravascular exchangeable protein exceeds that of plasma protein (Fig. 6). Thus in experiments in which whole labelled plasma was injected, plasma samples provide an underestimate of the average specific activity of the exchangeable protein pool, and the apparent pool size does not return to the initial value observed during perineal resorption. With the labelled albumin alone, however, specific activity is uniform in the intra- and extra-vascular compartments; pool-size estimations therefore appear to be accurate for this fraction, and indicate that the mass of exchangeable albumin increases by about 1 g./day during tumescence (Fig. 8).

Rates of synthesis of plasma protein in the baboon have been calculated in the conventional manner from plasma ^{131}I elimination curves (Sterling, 1951). The exponential rates of decay of plasma protein which have been deduced from the available data are somewhat arbitrary, especially during the phase

of perineal involution (see Figs. 2, 3 and 8); calculated rates of synthesis of plasma protein (Table 2) must therefore be regarded as approximate. The specific activity of globulin is not uniform throughout the exchangeable pool, even at 10 days after the injection of labelled plasma. Plasma protein decay curves are consequently influenced in these experiments not only by the rate of protein synthesis, but also by the return into the circulation of globulin of relatively high specific activity. The estimated rate of synthesis is therefore probably too low; however, since about 80 % of ^{131}I in labelled plasma is bound to albumin, the error is probably small. During perineal tumescence the apparent rate of synthesis exceeds that of catabolism calculated from ^{131}I excretion. During perineal involution the rate of decline of plasma specific activity is reduced and is slowest during the latter half of the luteal phase. The progressive decrease in the pool size that occurs during this time would tend to accelerate the decline of plasma specific activity if the mass of plasma protein synthesized per unit time were constant. There appears to be no doubt, therefore, that synthesis of plasma protein is reduced during perineal involution. At the same time the apparent rate of protein catabolism calculated from the excretion of ^{131}I rises above that observed during the first half of the cycle. However, the significance of this increase is uncertain in the animals which received iodinated whole plasma, since it might have resulted, in part, from the catabolism of globulin having a relatively high specific activity and derived from involuting perineal tissue.

SUMMARY

1. The menstrual cycle of the baboon is associated with marked alterations in the size and protein content of the perineal swelling. The distribution and turnover of ^{131}I -labelled plasma protein alters in step with the periodic growth and involution of this tissue.

2. During the follicular phase of the cycle the volume of the perineal swelling increases by about 3 l. and its protein content by approximately 60 g. After the intravenous injection of tracer doses of iodinated plasma, or albumin alone, the specific activity of circulating protein declines at an approximately constant exponential rate throughout perineal tumescence. Protein synthesis, calculated from plasma specific activity curves, exceeds catabolism estimated from the excretion of ^{131}I ; and the exchangeable protein pool increases in size.

3. Comparison of the specific activities of plasma and perineal tissue fluid, as well as the results of electrophoretic and ultracentrifugal analyses, indicates that the protein which accumulates in the tumescent perineum consists of extravascular

plasma protein which is probably lodged predominantly in connective-tissue ground substance.

4. The extravascular plasma protein of the perineum continues to exchange with circulating protein during growth of the perineal swelling. After equilibration the specific activities of plasma and perineal fluid albumin remain identical. Globulin fractions, on the other hand, attain a higher specific activity in perineal fluid, so that calculations of pool size based on the specific activity of total plasma protein are erroneously high.

5. During perineal involution the decline of the specific activity of plasma protein is very much slower than during the first half of the cycle, indicating a reduced rate of synthesis of plasma protein. At the same time, the size of the exchangeable plasma protein pool becomes progressively smaller.

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