

THE RELATION BETWEEN THE TISSUE
AND BLOOD PLASMA PROTEINS,
WITH SPECIAL REFERENCE TO THE PERINEAL TISSUE
OF THE BABOON (PAPO URSINUS).

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BY

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DECLARATION.

I hereby declare that this dissertation entitled:
The relation between the tissue and blood plasma
proteins with special reference to the perineal
tissue of the baboon (*Papio ursinus*), is my own
work and has not been previously submitted as a
dissertation or thesis for any degree.

Date: *27th February, 1958* *S. Shall.*

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SECTION A.

INTRODUCTION.

This study is directed towards an understanding of the tissue fluid and its regulation in the sex skin of the baboon, with particular reference to the composition of the blood plasma as a determinant of the composition of the tissue fluid.

The sex skin of the Chacma baboon lends itself particularly well to such an investigation because it swells under the influence of oestrogen and the tissue fluids accumulate there in great abundance during the first part of the menstrual cycle. The perineal tissue is a relatively homogenous connective tissue, consisting, like other connective tissues, of cells, fibres and tissue gel or tissue fluid.

However, the sex skin of the Chacma baboon is a specialised connective tissue insofar as the metabolism of the tissue fluid, the cells and the capillaries is rhythmically changed by the ovarian hormones. Unlike morphologically similar connective tissues in other parts of the body, the connective tissue of the sex skin undergoes an enormous expansion in volume during the follicular phase of the menstrual cycle, and regresses during the luteal phase. The response of the perineal connective tissue is due to the specific reactivity of the sex skin tissue, and possibly also to the composition of the perineal tissue fluid, which constitutes the immediate environment of the perineal tissue cells. The

constancy of the composition of the tissue fluid is a prerequisite for the continued existence of the cells. On the other hand, appropriate variations in the composition of the tissue fluid maintain the specific reactions of the perineal connective tissue. The constancy and variability of the tissue fluid are regulated by a diversity of factors, including local and distant cellular activity. Some of the physiological factors operative in this respect include diet and the endocrine, cardiovascular, urinary, alimentary and nervous systems. The blood plasma is an important source of materials entering the tissue fluid and therefore the composition of the blood plasma is a determinant of the composition of the tissue fluid.

A study of the plasma proteins in the female Chacma baboon has revealed a regular variation in plasma protein concentration during the menstrual cycle (Cohen, 1954 b). These variations in plasma protein concentration are associated with perineal hypertrophy and regression. The baboon sex skin therefore presents a concrete situation in which to investigate the relationship between the plasma proteins and the tissue proteins.

The comparison of the composition of the plasma proteins with that of the tissue fluid proteins will show a large increment in perineal proteins during perineal turgescence. Evidence will be presented to suggest that the protein accumulating in the enlarging sex skin of the female baboon is derived, in the first instance, from

the blood plasma. The data to be presented in this study, together with the information already available in the literature, will favour the view that the proteins in the turgescent sex skin are dependent on a rhythmical alteration in the rate of synthesis and catabolism, and in the distribution of the plasma proteins during the menstrual cycle. The accumulation of protein in the perineum will also necessitate a consideration of the changing reactivity of the tissues of the sex skin which, in the first part of the cycle, permits plasma proteins to accumulate and to be retained in the sex skin and thereafter to be liberated and discharged into the circulation during the luteal phase.

The absence of any detectable cyclic increment in the protein of the collagen fibres will be demonstrated.

Turning from the protein to the water of the perineum, it will be shown that the major portion of the increment in perineal mass is due to a local accumulation of water derived mainly from exogenous sources. It will be suggested that the presence of a polyelectrolyte gel, possibly containing an acidic polysaccharide, may provide a partial explanation, at least, of the water-retaining properties of the sex skin of the Chacma baboon.

The physiological factors regulating the composition and movement of the tissue fluids in the sex skin initiate a series of events unique among the connective tissues of the body. The specific mechanisms responsible for the cyclic swelling in the sex skin of

the baboon may not necessarily operate in other connective tissues of the body. Nevertheless, an understanding of the factors regulating the composition of the sex skin may serve to indicate the classes of physiological factors involved in regulating regional differences in the composition and movement of the tissue fluids in physiological and pathological states.

SECTION B.

MATERIAL AND METHODS.

1. The experimental animals.

This study has been conducted upon a group of female baboons belonging to a single species originally classified as *Papio porcarius*, renamed *Papio comatus* and now generally called *Papio ursinus* (Gillman and Gilbert, 1946). This animal is commonly known as the Chacma baboon.

Each animal of the colony is separately housed in open-air, wire-netted cages which are about six feet six inches high and five feet square and is provided with a shelter, heated during the winter months. Animals maintained under such conditions are exposed to considerable fluctuations of atmospheric temperature, humidity, radiation and wind velocity. Despite the very marked seasonal fluctuations characteristic of the climate of Johannesburg, the baboons are in excellent physical condition and very rarely show signs of illness.

The baboons are fed twice a day at about 10.00 a.m. and 3.30 p.m. The first meal is cooked porridge containing 71 percent maize, 12 percent minced liver, 10 percent skimmed milk powder, 6 percent fat and 1 percent sodium chloride. The second meal is mixed vegetables and fruit, and always includes potatoes and some green vegetable. The quantity of food provided is not accurately measured, but the edible remnants remaining in the cage at the end of the day are

evidence that appetite has been satisfied with the minimum waste of food. The diet alters with seasonal variations in fruit and vegetable production. Although the diet is restricted the animals can exercise a measure of selection.

The baboons are maintained under artificial conditions. The imposed patterns of diet, physical activity and social behaviour are very different from those of the animal in the veld. Nevertheless, certain minimal conditions have been satisfied which allow the captive baboon to maintain that sensitive physiological process which regulates the menstrual cycle and fertility. The variations in perineal tissue and plasma protein composition which will be recorded for this group of captive baboons can therefore be regarded as characteristic of the species in general.

Fluctuations in the size of the perineal swelling of the adult female baboon were established by daily measurement of the distance between ischial callosities (Figures 1 and 8). From these measurements the menstrual cycle was divided into two parts (Gillman and Gilbert, 1946). The first, referred to as the period of turgescence, begins on the first day of menstruation and is characterised by progressive enlargement of the perineal swelling. The subsequent deturgescent phase is associated with involution of the perineal swelling and is terminated by the onset of the next menstrual bleeding.



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Figure 1. Two baboons showing (1) a perineum in a state of maximal turgescence and (2) a perineum at deturgescence.

2. The blood sample.

Some conclusions of the present investigation will be based on the assumption that the protein concentration of a small blood sample is representative of the protein concentration throughout the total circulating plasma and in particular of the protein concentration in the capillaries of the perineal tissue. Two factors may alter the protein concentration of a specific sample and so render it unrepresentative. These are the technique of collection and storage of the blood sample and the regional differences in plasma protein concentration.

Protein determinations are generally carried out on blood from a peripheral vein. Numerous investigations have indicated that the protein content of the sample will be affected by the technique of venepuncture, blood collection and storage. The factors which have received attention in regard to their influence on protein concentration are:-

- (1) venous stasis (Böhme, 1911; Yamaguchi, 1927; Schwensen, 1924; Peters, Eisenman and Bulger, 1924; Vahlquist, 1941; Lange, 1946);
- (2) haemolysis (Rowe, 1917; Lange, 1946);
- (3) use of anti-coagulants (Eisenman, 1927; Schmidt, 1935; Marx, 1935; Lange, 1946; Wintrobe, 1946; McLain, Ruhe and Pastorious, 1949);
- (4) storage (Cohen, 1954 b).

In view of these investigations the following routine was adopted.

The blood was collected in a dry syringe from a peripheral vein in the horizontally extended arm or leg. The vein was compressed proximal to the needle for five to thirty seconds and released immediately after blood flow commenced. The blood was immediately added to a tube containing solid anti-coagulant (Wintrobe, 1946) and inverted several times. The blood was stored at 4° C. until it was used, which was not more than four hours after collection.

The extent to which plasma protein concentration may vary in different regions of the body has not been fully characterised. Some studies (Pein, 1932; Achard, Bariety, Codounis and Hadjigeorgies, 1934; Shida, 1939 a, 1939 b; Gerbi, 1951) indicate that the serum protein level of renal blood may exhibit an appreciable arterio-venous difference, the direction and extent of which alters under changing biological conditions. A long-standing observation, recently confirmed, indicates that the plasma fibrinogen level may be significantly higher in arterial than in venous blood (Dastre, 1893; Schultz, Nicholes and Schaeffer, 1925), and that a concentration gradient exists between the blood of the pulmonary artery and vein (Cohen, 1951).

Protein analysis of peripheral venous blood does not provide precise information concerning the protein level in different parts of the circulation. In view of this lack of precise information the

concentration of plasma proteins in the peripheral limb vein has been assumed to be identical with that throughout the body and in the capillaries of the perineum.

3. The tissue.

(a) The tissue sample.

A sample of perineal tissue was removed from the animal under light general anaesthesia induced by the intravenous administration of sodium pentothal (10 mg. per Kg. body weight) and maintained with anaesthetic ether.

The perineum was then washed with warm water and dried. A small area about midway between the anus or vagina and the lateral margin of the swelling was swabbed with 95 per cent ethanol. From this area a wedge of tissue about one inch by half an inch and about one and a half to two inches deep was excised.

The wound was closed with two or three deep catgut sutures, apposing the cut surfaces and arresting the bleeding. The wounds healed in about three weeks. More rapid healing was observed during the luteal phase of the menstrual cycle than during the follicular phase, probably because there was less mechanical tissue tension on the wound. The hormones present may also have aided the healing process.

A thin slice of tissue was excised from each sample at right angles to the surface and fixed in 10 per cent formalin for histological

examination.

The dermis and epidermis were separated from the deeper white connective tissue and only the latter portion was used for analysis. The deeper tissue was transferred to a tared watch glass and weighed with the minimum loss of time. Aliquots were immediately removed for the various analytical procedures.

Two factors could have influenced the composition of the tissue samples:-

- (1) the operative procedures;
- (2) regional differences in tissue composition. There is no information available to indicate in what manner these two factors influence tissue composition.

(b) Fractionation of the tissue.

The tissue sample was tied to the cork of a centrifuge tube and centrifuged for five minutes at 600xG. A fluid, which sometimes contained a jelly, settled at the bottom of the tube. A solid residue remained attached to the cork. This fractionation is shown schematically in Figure 4 (1).

(c) Mass of perineal swelling.

The total volume of the perineal swelling was estimated in the following way. The animal was anaesthetised as previously described. The hair bordering on the perineal swelling was cropped

short. The swelling itself was washed well with warm water and soap. The skin was dried and oil was smeared on all the edges and crevices where the plaster cast would be likely to adhere. Wet plaster of Paris bandages were wrapped around the entire swelling including the clitoral lobe. In this way a cast of the entire sex skin was obtained (Figure 2). The bandages were allowed to dry and harden slightly and were then gently removed. The perineum was washed with warm water, cleaned and dried. The tissue sample was then excised as previously described. The cast was labelled and allowed to dry completely. The inside of the cast was impregnated with molten paraffin wax. The volume of the cast was measured by adding water until it was full. Five estimations were made and the average was recorded as the volume of the cast. The volume of the cast was assumed to be equal to the volume of the perineal swelling.

Replicate estimations of the volume of one cast yielded an average of 180 ml. with a range from 150 ml. to 200 ml. In another case an average of 1580 ml. with a range from 1500 to 1680 ml. was found. Thus in any particular comparison a variation of under 20 per cent was not regarded as significant.

Dissection of the perineum has revealed that the reactive tissue is bounded by a fascial layer which is relatively superficial in relation to surrounding structures (Cohen, 1954 b). Nevertheless, the exact extent of reactive tissue was not determined in each estimation and this might have introduced a serious error. Further,

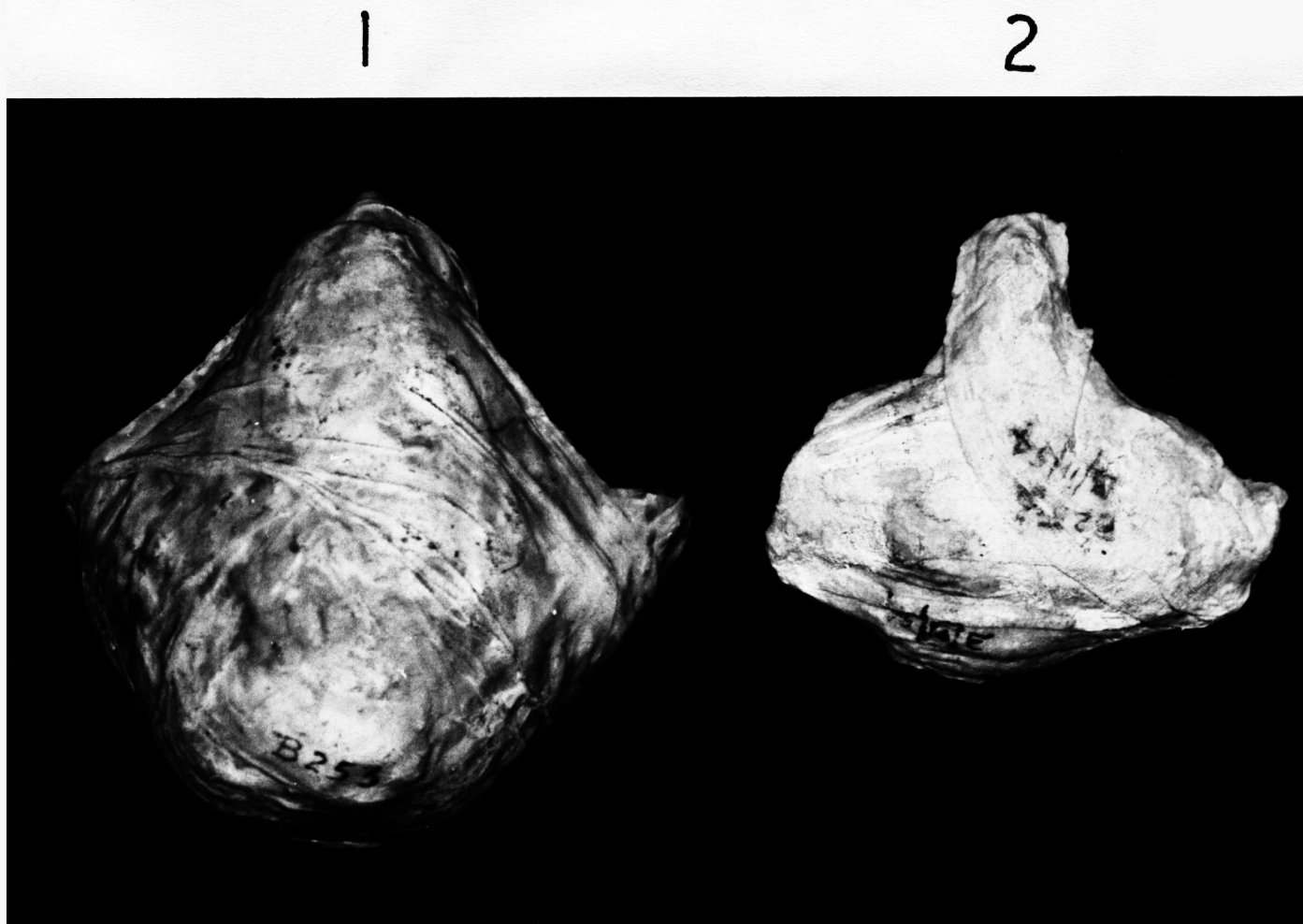


Figure 2. Plaster of Paris casts of the (1) maximally expanded and (2) resting sex skin.

the irregular shape of the swelling might not always have been reproduced accurately.

Direct gravimetric measurement would have yielded the most accurate assessment of the perineal mass. Unfortunately, this was not possible. Nor was it possible to estimate the specific gravity with any degree of accuracy with the small quantity of tissue available. It was therefore decided to assume an arbitrary constant specific gravity of 1.1, which was in most cases likely to be nearer to the real value than 1.0.

The total mass of the perineum was calculated by multiplying the measured volume by 1.1. A variation of 20 per cent or less in the total perineal mass as estimated by this method was not regarded as significant.

(d) Water content of perineal tissue.

The classical methods of estimating water concentration are extremely easy but not correspondingly accurate. The technique of choice in this study was a method of high reproducibility rather than one of extreme accuracy.

The weighed, wet tissue samples were dried to constant weight in a dry air oven at 105° C. To achieve a fair degree of drying a temperature of more than 100° C. was found necessary. Temperatures above 106° C. led to considerable charring and combustion of the tissue. 105° C. was the highest temperature that could be used without any charring.

The amount of water evaporated depends on the temperature. All non-chemically bound water appears to have been estimated because higher temperatures led to combustion of the tissue, which usually only occurs after all free water has been evaporated.

Different samples of the same wet tissue gave results within 2 per cent of one another.

4. Protein analysis.

(a) General.

At the present time, apart from proteins having unique measurable properties such as the enzymes, quantitative protein analysis depends either upon the estimation of some element or group contained in the molecule or on the measurement of certain physical properties of protein solutions. The chemical methods are based on the estimation of nitrogen, specific amino acids such as tyrosine or hydroxyproline, peptide bonds and formol binding groups. In addition, the presence of certain constituents can be used to measure proteins; for example, iron and iodine in haemoglobin and thyroglobulin respectively. Physical methods of analysis include measurement of density (or specific gravity), refractive index, viscosity, surface tension, absorption of radiation, electrical conduction properties and rotation of polarised light.

All methods of quantitative protein determination are ultimately based on the estimation of a chemical element or group having an approximately known distribution within the protein molecule. Errors

result from the technical difficulties of the analytical method, from inadequate knowledge of the percentage composition of the particular protein regarding the element analysed and from interference by non-protein constituents of the fluid or tissue examined. In practice, nitrogen is used to measure plasma and tissue proteins. The virtually unique presence of the amino acid hydroxyproline in collagen can be used to estimate the concentration of this protein.

The extraordinary complexity of proteins and the difficulty of separating, purifying and drying these compounds have prevented the successful elaboration of accurate, direct, gravimetric methods of quantitative protein analysis (Kirk, 1947). Consequently, all techniques of protein estimation remain inherently inaccurate. However, a physiological study of the plasma and tissue proteins does not require a technique with a high degree of absolute accuracy, but one of consistent reproducibility.

Kjeldahl's method of measuring nitrogen quantitatively is the most widely applied technique for the determination of protein in all biological material, including blood plasma and tissues. The micro-Kjeldahl, biuret and amino acid analysis techniques adequately fulfil the requirement of consistent reproducibility and have therefore been adapted for plasma and tissue protein analysis in the present investigation.

(b) Nitrogen estimations.

Nitrogen determinations were performed by the micro-Kjeldahl method (Hiller, Plazin and van Slyke, 1948). In addition to the

mercury catalyst a few milligrams of the following selenium mixture was added to the digestion tube to aid the liberation of nitrogen from the proteins.

Selenium mixture:

0.34 g. sodium selenate (Na_2SeO_4).

20 g. copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$).

80 g. potassium sulphate (K_2SO_4).

Investigation of the nitrogen content of human plasma protein has led to divergent results. Observed variations in the nitrogen content of plasma or serum protein result both from lack of uniformity of analytical procedures and from differences in moisture and ash content of the proteins analysed (Hiller, Plazin and van Slyke, 1948). Recent investigations in which these factors are standardised reveal a fairly uniform nitrogen content of total protein approximating to 16 per cent.

The available data indicate that different species are alike in regard to the nitrogen content of plasma protein (Block, Darrow and Cary, 1934). In the absence of specific information concerning the baboon it appeared likely that a negligible error would result from the assumption that nitrogen constitutes 16 per cent of plasma protein. A somewhat larger error would be involved by assuming that tissue proteins on the average contain 16 per cent nitrogen.

Perineal tissue in rhesus monkeys contains between 8 and 27 per cent non-protein nitrogen (Ogston, Philpot and Zuckerman, 1939).

Thus, working on the assumption that all the nitrogen in the perineum of the baboon is derived from protein, the maximum error incurred would not be more than 30 per cent. The magnitude of this error is small compared with the changes occurring during the menstrual cycle. Although some of the perineal nitrogen in fact is derived from non-protein sources, it was deemed adequate for the purposes of this study, to regard all the nitrogen in the sex skin as derived from protein.

(c) Biuret technique.

The biuret technique has been used to estimate the concentration of soluble protein. The biuret method has the advantage that the reaction on which it is based is given by few of the probable non-protein constituents of plasma. In addition all proteins, including serum albumin and globulin obtained from healthy subjects, give almost exactly the same biuret colour per unit mass of protein.

Total soluble protein, soluble albumins and globulin concentration were estimated by the biuret method of Weichselbaum (1946) as modified by Gornall, Bardawill and David (1949). Readings were made in a Coleman spectrophotometer at 540 m μ 30 minutes after adding the biuret reagent (Figure 3). The significant advantage of this method over techniques previously described is the use of a stable "one-piece" biuret reagent which maintains optical clarity when mixed with clear plasma.

(d) Salt fractionation.

Soluble protein was fractionated into soluble albumin and insoluble globulin by precipitation with 28 per cent sodium sulphite (Wolfson, Cohn, Calvary, and Ichiba, 1948). The albumin concentration was determined by the biuret method. The globulin concentration was calculated by deducting the albumin concentration from the total protein concentration. This system of fractionation does not separate electrophoretically pure components. The albumin obtained by salt fractionation corresponds to the albumin plus alphaglobulin fractions as measured electrophoretically (Cohen, 1954 b).

The methods of protein analysis thus far described contain two sources of inaccuracy:-

- (1) accidental errors due to faults in the execution of the technique;
- (2) systematic errors inherent in the method.

In all but 5 per cent of cases a single total soluble protein determination performed according to the biuret method as applied in this study will give a value within 0.34 g. per cent of a duplicate estimation. Accordingly, in 95 per cent of instances, deviations in total soluble protein concentrations greater than 0.34g.per cent cannot be attributed to accidental analytical error.

In 95 per cent of cases a single determination of albumin concentration will be within 0.30 g. per cent of a duplicate

determination, and a single globulin analysis will be within 0.44 g. per cent of a duplicate. Again, in all but 5 per cent of instances, deviations in albumin concentration greater than 0.30 g. per cent and in globulin concentration greater than 0.44 g. per cent. cannot be attributed to accidental analytical error.

The use of a single, standard calibration curve (Figure 3) assumes an unchanging relationship between the biuret reactivity, nitrogen content and protein mass in all the samples analysed. The nitrogen-biuret ratio of soluble serum proteins is disturbed in certain severe pathological conditions (Kingsley and Behrend, 1949; Kingsley and Terzian, 1949). On the other hand, studies mainly on normal sera obtained from human subjects and from dogs, revealed a maximum deviation of 0.28 per cent between Kjeldahl and biuret determinations (Weichselbaum, 1946; Gornall, Bardawill and David, 1949). The correspondence between the Kjeldahl and the biuret methods of plasma protein determination have been previously established in the baboon (Cohen, 1954 b).

The error inherent in the biuret technique therefore is small in relation to the magnitude of the accidental error.

(e) Electrophoresis.

It is convenient to distinguish between boundary electrophoresis and zone electrophoresis. In the former method the movement and separation of boundaries is observed by optical or analytical methods.

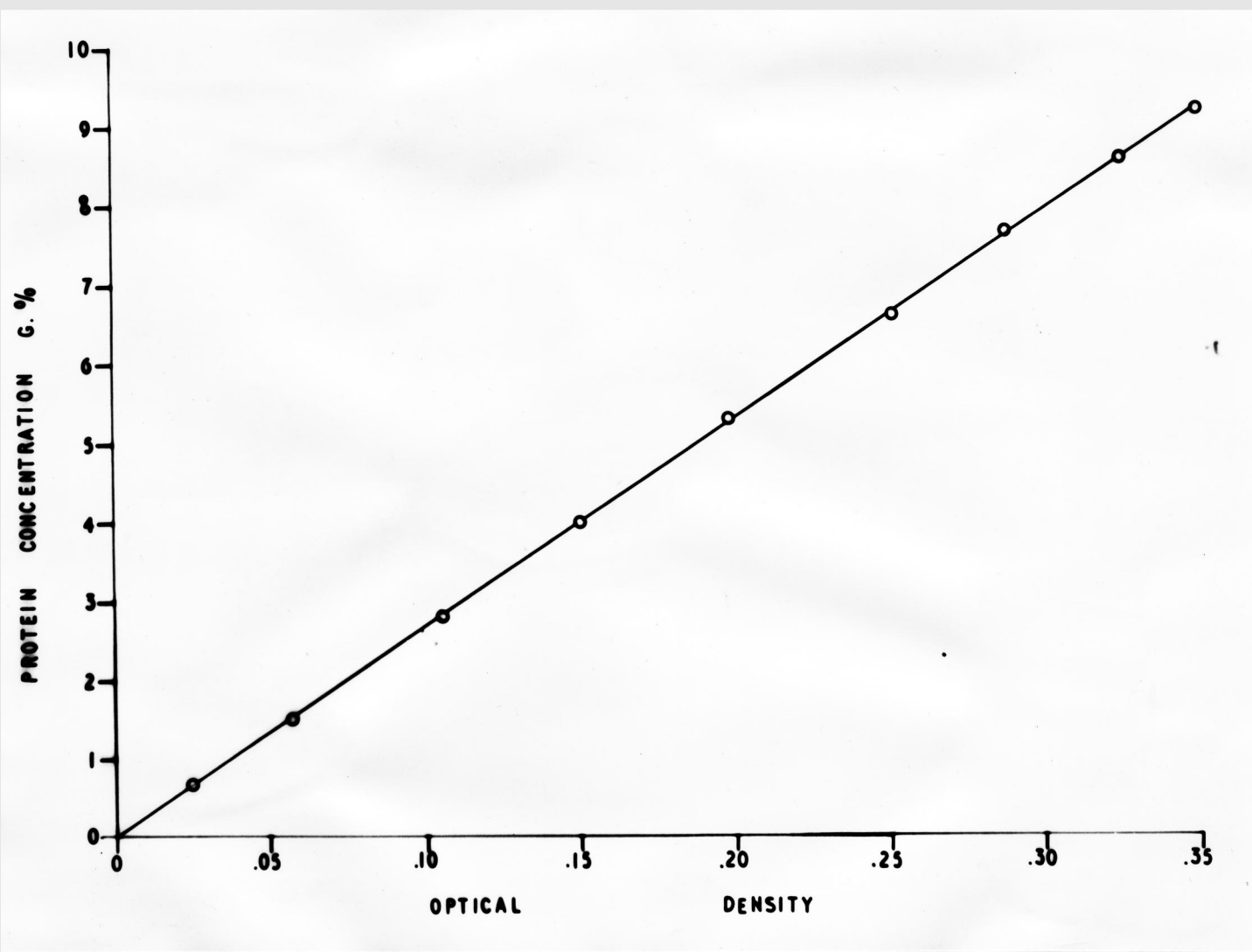


Figure 3. Standard curve of protein concentration and optical density used in the Biuret method.

In the latter the components studied migrate as separate zones, which is possible only if they are stabilized against convection by some sort of supporting medium, e.g. filter paper, starch, glass powder, silica, agar gel or cellulose.

The chief advantages of zone electrophoresis compared with the boundary procedure are that it is possible to obtain complete separation into zones of different migration and not merely a boundary separation of overlapping zones. Also, the so-called boundary anomalies interfere less in zone electrophoresis. In addition, zone electrophoresis requires only minute quantities of material and can be performed with simple and inexpensive apparatus.

These advantages of zone electrophoresis are gained, however, at the sacrifice of the greater accuracy of the boundary method. The supporting medium necessary in zone electrophoresis introduces new variables which influence the results in a way which is difficult to control.

Filter paper strips have recently been widely used with notable success as supporting media for zone electrophoresis (filter paper electrophoresis). Recent contributions have converted this simple procedure into a very important tool in biological research (Wieland and Fischer, 1948; Durrum, 1950, 1951 a, 1951 b; Cremer and Tiselius, 1950; Kunkel and Tiselius, 1951).

Comprehensive reviews of zone electrophoresis have been published by Tiselius and Flodin (1953) and Kunkel (1954). A brief review of

filter paper electrophoresis has been published by Lederer (1951). Grassmann and Hannig (1952) have written a detailed discussion of the method and of its application to the electrophoretic analysis of blood serum.

In this investigation the basic apparatus of Kunkel and Tiselius (1951) was employed.

Procedure:

Two spots of plasma, each of 0.01 ml., were placed two inches apart on a line (the origin) four inches from the centre on the cathode end of the Whatman No. 1 paper strips. The paper strips were arranged symmetrically along a glass plate. Another glass plate was very carefully apposed. The two plates were sealed by forcing petrolatum jelly between them in order to fill the space between the edges of the paper and of the plates. One litre of phosphate buffer, pH 7.5, $\mu = 0.1$ was placed in each vessel containing the electrode. The connecting tube between the vessels was opened and the two liquid levels were allowed to equilibrate. After switching on the current, the connecting tube was closed by means of a clamp. The potential difference between the electrodes was set at 300 volts, giving a potential gradient of 5.9 volts per cm. along the strips. Under these conditions 2.8 milli-amperes current flowed through the circuit. The potential was applied for a measured period of time, usually 22 hours, at room temperature. There was no obvious increase in the temperature of the plates.

After the current was switched off the two glass plates were carefully separated and the filter paper strip was air dried. It was then stained for 2 to 3 minutes in a 0.5 per cent aqueous Bromophenol Blue solution saturated with mercuric chloride. This treatment coloured the entire paper a bright yellow. The protein patterns were then differentiated by immersing the strips for 15 minutes in each of three changes of 0.5 per cent acetic acid.

After differentiation the two patterns stood out yellow-green on a white background, and were then separated from each other. One was passed through ammonia vapour which neutralised the acid dye, transforming it into the more intensely coloured blue dye. This pattern was kept for record purposes.

The other pattern was divided into consecutively numbered 0.5 cm. segments starting from the origin. The dye in each segment was eluted in 2.5 ml. of 0.01 N sodium hydroxide for thirty minutes. The optical density of the dye in the eluate from each individual segment was measured in a Beckman Model DU spectrophotometer at 595 m μ in 1 cm. corex cells with 0.01 N sodium hydroxide as the blank. The optical density of the dye from each segment was plotted against the distance of the segment from the origin (Figures 6 and 7).

When the electrophoretic patterns of plasma separated by free boundary electrophoresis in the classical Tiselius apparatus and by the method described above were compared, they showed a qualitative

similarity. The proportions of the components varied within 7.5 per cent of one another.

One sample of plasma was separated repeatedly by the method described and the patterns obtained were similar to one another; the proportions of the components varied within 2.0 per cent of the mean.

The quantity of Bromophenol Blue dye bound by denatured proteins on filter paper is dependent on the nature and degree of denaturation, the time of staining and the amount of rinsing (Jencks, Jetton and Durrum, 1955). The electrophoretic method as described above cannot therefore be used quantitatively with any degree of accuracy. On the other hand, it is suitable for recognising similarities and differences in the composition of protein solutions. Its use in this inquiry is primarily for this latter purpose.

(f). Estimation of soluble and insoluble nitrogen.

The constituents of the perineal tissue were divided into two fractions, one soluble and one insoluble in 0.9 per cent sodium chloride (Figure 4 (2)).

The wet tissue sample was triturated and washed three times with normal saline. The saline extracts were centrifuged until they yielded a clear solution. This clear saline extract contained the soluble material. The precipitate and residue remaining in the centrifuge tube was considered as insoluble nitrogen-containing

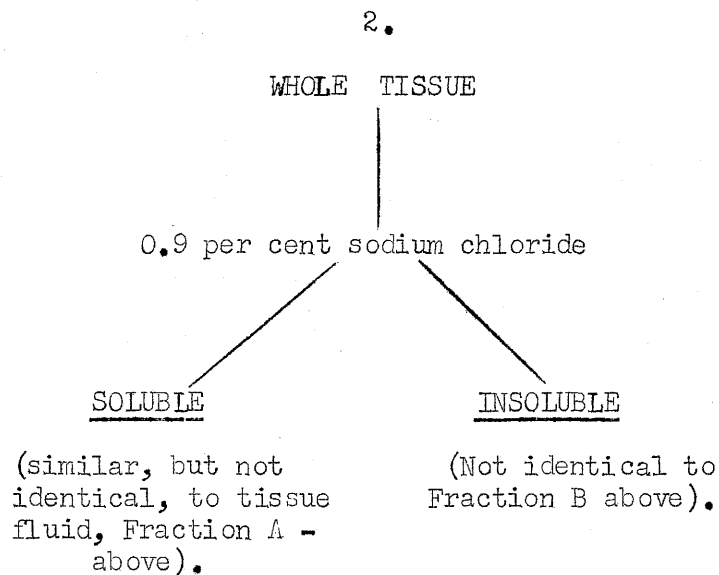
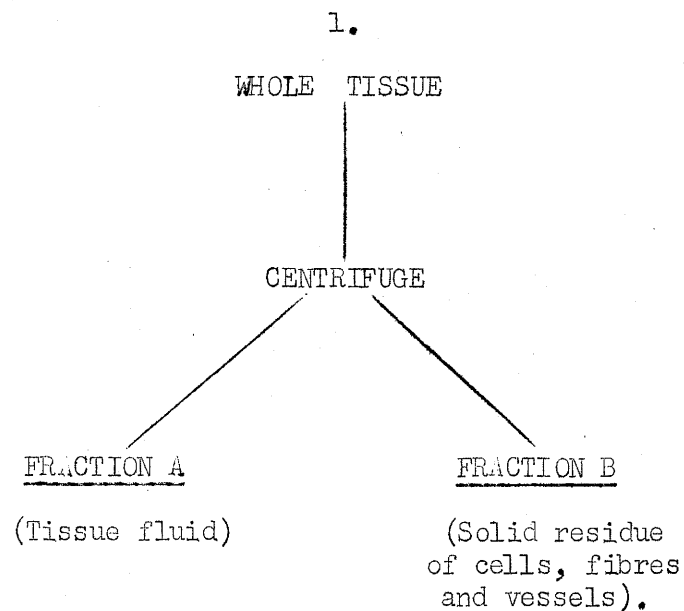


Figure 4. Summary of fractionation procedure. Perineal tissue was fractionated by (1) centrifugation and (2) solubility in normal saline.

material. The respective nitrogen contents were determined by the micro-Kjeldahl method.

Because variations in the fineness and completeness of trituration were expected, triplicate estimations were made. The average deviation of a particular estimation from the mean was ± 0.60 g. soluble nitrogen per 100 g. original wet tissue and ± 0.42 g. insoluble nitrogen per 100 g. original wet tissue.

The suitability of normal saline as a solvent for proteins which are soluble in tissue fluid is undetermined. It was, therefore, not possible to assess the accuracy of the method. Further, the process of trituration may have altered the solubility of some of the tissue proteins, but the error from this source could not be gross.

A certain proportion of the soluble nitrogen was probably estimated together with the insoluble nitrogen. Soluble nitrogenous material may have been adsorbed onto the particles and deposited during centrifugation. The soluble nitrogen was possibly underestimated and the insoluble nitrogen overestimated.

(g). Protein hydrolysis.

The protein hydrolysates used in the amino acid analyses were prepared in the following way.

The weighed, dried tissue was placed in a clean, dry, pyrex glass tube. 1 ml. of 6 N hydrochloric acid was added for every 50 mg. of dried protein. The tube was sealed and incubated in a dry air oven

at 120° C. for 16 hours.

The two variables, temperature and time, were fixed empirically. Temperatures of 140° C. and 150° C. yielded lower recovery values for any period of incubation than did 120° C. The loss of tyrosine was particularly marked at the higher temperatures.

At 120° C., 16 hours incubation gave the maximum yield of hydroxyproline from several tissue samples. The extent of the amino acid destruction was tested by the incubation of crystalline amino acids. 96 per cent of the hydroxyproline and 90 per cent of the tyrosine was recovered after 16 hours incubation at 120° C.

After incubation the pyrex tubes were opened and the solutions filtered into volumetric flasks. The solutions were made up to volume. Aliquots of ~~these~~ diluted solutions were neutralised with sodium hydroxide and used for amino acid determination.

(h). Hydroxyproline estimations.

Histologically baboon perineal tissue is a loose connective tissue. In the sex skin collagen fibres can be detected by histological methods. From collagen fibres proteins commonly known as collagens can be extracted. These proteins contain a relatively high content (± 14 per cent) of the amino acid hydroxyproline (Tristram, 1953). This amino acid is virtually absent from all non-collagenous proteins. Its presence and behaviour can therefore be utilized as a measure of the amount of collagen in the tissue at any given time.

Hydroxyproline can be estimated in a protein hydrolysate by oxidative decarboxylation to indole derivatives. The latter substances can then be conjugated with p-dimethyl-amino-benzaldehyde (Ehrlich's reagent) to give a characteristic orange-red colour. The concentration of hydroxyproline is proportional to the optical density of the colour produced (Figure 5).

The actual method used was that of Neuman and Logan (1950). A standard curve was prepared using known concentrations of hydroxyproline solution (Figure 5). Crystalline hydroxyproline was kindly supplied by Dr. Winter of the Poliomyelitis Research Laboratories, Rietfontein.

Replicate determinations on a standard solution containing 20 μ g. of hydroxyproline per ml. varied between 19.0 and 21.5 μ g. per ml. Replicate determinations on a standard solution containing 5 μ g. hydroxyproline per ml. varied between 4.5 and 5.4 μ g. per ml. A difference of more than 20 per cent between two determinations was therefore regarded as significant.

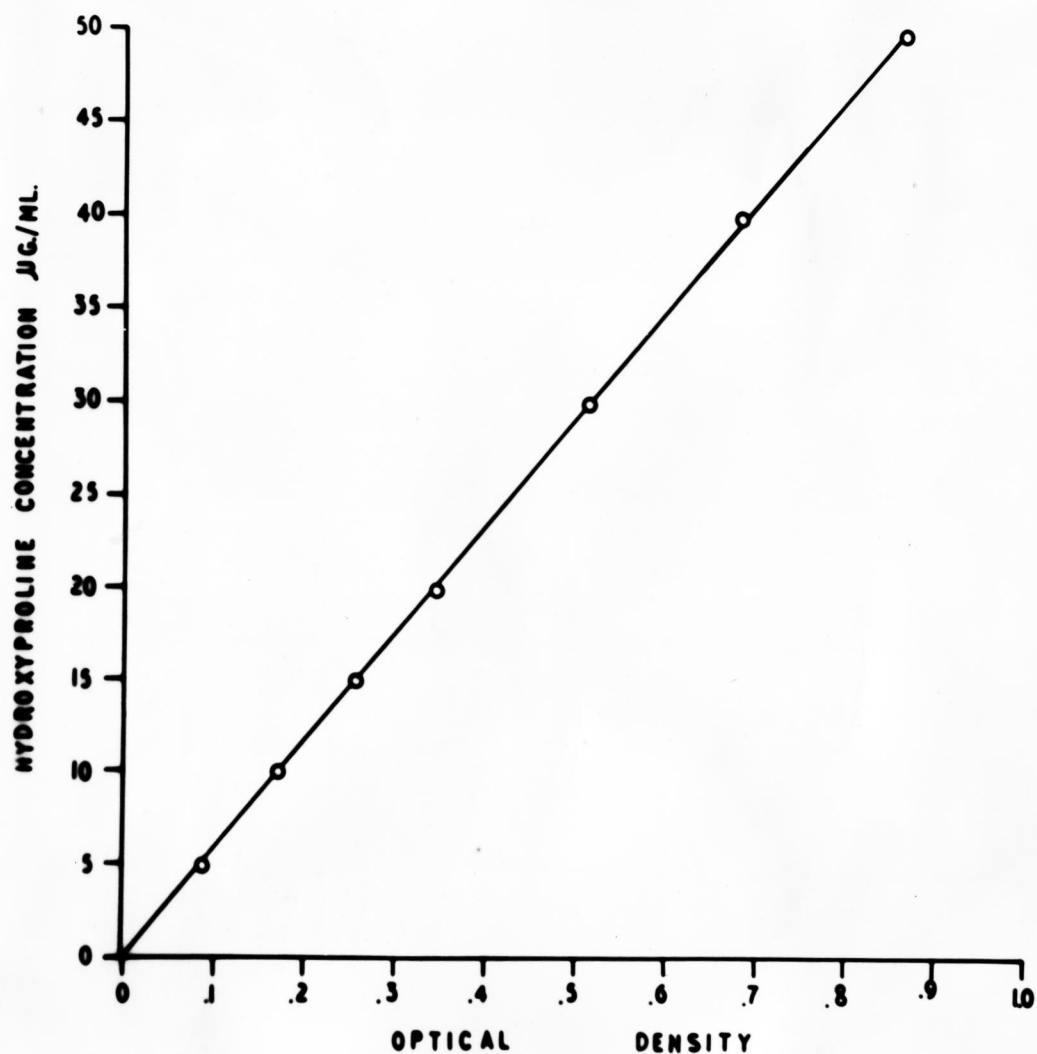


Figure 5. Standard curve of hydroxyproline concentration and optical density using Ehrlich's reagent.

SECTION C.

EXPERIMENTS AND RESULTS.

1. Introduction.

In this section it will be demonstrated that the proteins of the perineum increase in mass as the tissue turgescens. The partition of these proteins between the tissue fluid and the formed elements will be described.

The increase in the mass of perineal proteins was mainly due to an increase in the volume of the perineum, and not to changes in protein concentration. Therefore, the variations in volume and mass of the perinea are described.

Table 1 indicates the volumes and masses of the perinea of six baboons at the two stages of the perineal cycle. The flaccid perinea in the several baboons showed different volumes. For example, B. 316 had a volume of 130 ml. and B. 354 a volume of 250 ml. By contrast, the volume of the turgid perineum in B. 362 was 1120 ml., which was a remarkably large increase in the volume of the perineum. The increase in volume in the other animals was also remarkably large, but in each case wide individual variations were evident.

Throughout this study wide individual variations were found. Since all the animals studied were mature, adult females, the observed inter-individual variations were all manifestations of individuality. One baboon, for example, B. 354, possessed an exceptionally large turgescens perineum, over 3.5 litres in volume.

TABLE 1.

Volume and mass of the perinea of six baboons
at maximum turgescence and at deturgescence

Animal Number	Perineal Volume ml.		Perineal mass grams.		Ratio of Turg./ Deturg. mass. $\frac{x}{y}$	Increase in mass grams x-y
	Turg.*	Deturg.*	Turg. x	Deturg. y		
B. 362	1120	145	1230	159	7.7	1070
B. 344	1400	215	1540	236	6.5	1300
B. 316	1440	130	1590	143	11.1	1440
B. 253	1540	170	1690	187	9.0	1500
B. 319	1780	220	1950	242	8.1	1710
B. 354	3670	250	4040	275	14.7	3770

*In this and subsequent tables, Turg. = turgescent and

Deturg. = deturgescent.

2. The nitrogen content of the perineum.

(a) Total nitrogen.

The absolute total nitrogen content of the sex skin of all six baboons increased markedly as the perineum became turgid (Table 2). The perineum of B. 316 showed an increase of total nitrogen of 7.7 g. The sex skin of B. 354 increased in total nitrogen by 23.2 g. The total nitrogen in the flaccid perineum varied from 1.9 g. (B. 316, B. 362) to 5.2 g. (B. 319) and rose to between 9.6 g. (B. 316) and 13.8 g. (B. 362). In B. 354, however, the total nitrogen content of the turgescient perineum was 26.5 g. Increases of from about 4 to 8 times the resting values were observed in the several baboons.

A distinct dilution of the total perineal nitrogen during perineal turgescence was observed in three animals, B. 319, B. 316 and B. 344.

The nitrogen of the perineum was divided into nitrogen soluble in 0.9 per cent sodium chloride and nitrogen insoluble in 0.9 per cent sodium chloride (Figure 4 (2)). The soluble nitrogen included the soluble nitrogen of interstitial fluid, cells and fibres, and intravascular plasma. Because perineal tissue is relatively avascular and acellular, a large proportion of the soluble nitrogen was probably derived from interstitial fluid. The exact proportion however, was not determined.

TABLE 2.

Total nitrogen concentration and mass in the perinea of six baboons at maximum turgescence and at deturgescence.

Animal Number	Nitrogen concentration		Mass of nitrogen		Increase in mass of nitrogen grams x - y
	g. per cent Turg.	Deturg.	grams Turg. x	Deturg. y	
B. 316	0.61	1.36	9.6	1.9	7.7
B. 344	0.84	2.15	12.9	5.1	7.8
B. 319	0.70	2.14	13.7	5.2	8.5
B. 253	0.89	1.06	10.6	2.0	8.6
B. 362	1.12	1.19	13.8	1.9	11.9
B. 354	0.66	1.20	26.5	3.3	23.2

(b) Soluble nitrogen.

The mass of soluble nitrogen in the perinea of the six baboons increased during the follicular phase of the menstrual cycle (Table 3). The mass of soluble perineal nitrogen in B. 354 rose from 1.3 g. to 16.6 g., an increase of 15.3 g. The remaining five animals were grouped more closely together, evincing increases of from 6.3 g. (B. 362) to 9.1 g. (B. 319). The increase of soluble nitrogen was roughly proportional to the degree of perineal expansion. In the

two extreme cases, the soluble nitrogen mass in the perineum increased 6.9 (B. 344) and 15.2 (B. 316) times its resting value.

TABLE 3.

The mass of soluble nitrogen in the perinea of six baboons at maximum turgescence and at deturgescence.

Animal Number	Concentration of soluble nitrogen. g. per cent		Mass of soluble nitrogen. grams		Ratio of Turg./ Deturg. mass. $\frac{x}{y}$	Increase in mass of soluble nitrogen. grams $x - y$
	Turg.	Deturg.	Turg. x	Deturg. y		
B. 362	0.56	0.37	6.9	0.6	11.5	6.3
B. 253	0.45	0.41	7.6	0.8	9.5	6.8
B. 316	0.48	0.35	7.6	0.5	15.2	7.1
B. 344	0.58	0.56	8.9	1.3	6.9	7.6
B. 319	0.54	0.57	10.5	1.4	7.5	9.1
B. 354	0.41	0.41	16.6	1.3	12.8	15.3

The concentration of soluble perineal nitrogen was remarkably constant throughout the menstrual cycle (Table 3, columns 2 and 3). Inter-individual differences in concentration were minimal. These two observations taken together suggest a specific regulation of the

concentration of soluble perineal nitrogen. The concentration of soluble nitrogen at maximum turgidity varied from 0.41 (B. 354) to 0.58 (B. 344) g. per cent, and during perineal rest, from 0.35 (B. 316) to 0.57 (B. 319) g. per cent. The constancy of the concentration of soluble nitrogen indicates that the fluid which entered the perineum possessed a concentration of soluble nitrogen similar to that of the perineum itself. In other words, the fluid that entered the perineum contained about 0.5 g. per cent soluble nitrogen or about 3 g. per cent of soluble protein.

(c) Insoluble nitrogen.

A small apparent variation was observed in the mass of insoluble nitrogen in the perineum during the menstrual cycle (Table 4). A part of this apparent increase was probably due to the adsorption of soluble nitrogen to insoluble particles during centrifugation.

In 4 of the 6 animals there was a significant increase in insoluble nitrogen concentration as the swelling decreased in size. In these 4 animals the apparent increase in insoluble nitrogen mass was 0.5, 0.6, 0.6, and 1.6 g. In the other 2 baboons, B. 253 and B. 362, a significant difference was not detectable between the concentrations of insoluble nitrogen during perineal rest and at maximum turgidity. These two animals therefore showed an apparent increase in insoluble nitrogen mass as the tissue became swollen. The analytical method used was possibly not sufficiently accurate to estimate such changes as occurred. These determinations therefore

suggest that there was no increase in the mass of insoluble nitrogen, but more accurate methods of estimation are necessary before a definite conclusion can be reached. Any increase in insoluble nitrogen which might have occurred was relatively small compared with the increase in soluble nitrogen (Compare Tables 3 and 4).

TABLE 4.

The mass of insoluble nitrogen in the perinea of six baboons at maximum turgescence and at deturgescence.

Animal Number	Concentration of insoluble nitrogen.		Mass of insoluble nitrogen.		Increase in mass of insoluble nitrogen. grams x - y
	g. per cent. Turg.	Deturg.	grams Turg. x	Deturg. y	
B. 319	0.15	0.99	2.9	2.4	0.5
B. 316	0.10	0.71	1.6	1.0	0.6
B. 344	0.19	0.99	2.9	2.3	0.6
B. 354	0.09	0.72	3.6	2.0	1.6
B. 253	0.17	0.44	2.9	0.8	2.1
B. 362	0.42	0.59	5.2	0.9	4.3

The total, soluble and insoluble nitrogen concentrations were separately estimated. The approximation of the sum of the soluble and insoluble nitrogen fractions to the total nitrogen mass is therefore an indication of the accuracy of the three estimations. The wide variation in the extent of the recovery (72.7 to 112.8 per cent) is shown in Table 5. The lowest recovery, 72.7 per cent,

TABLE 5.

The sum of the increments of insoluble and soluble nitrogen masses in the tumescent perinea of six baboons, expressed as a percentage of the increase in total nitrogen.

Animal Number	Nitrogen increment grams			$\frac{\text{Soluble} + \text{insoluble}}{\text{Total}} \times 100$ Percentage
	Soluble	Insoluble	Total	
B. 354	15.3	1.6	23.2	72.7
B. 362	6.3	4.3	11.9	89.2
B. 344	7.6	0.6	7.8	105.2
B. 316	7.1	0.6	7.7	100.0
B. 253	6.8	2.1	8.6	103.4
B. 319	9.1	0.5	8.5	112.8

occurred in B. 354, in which case a larger experimental error was involved because of the exceptionally great size of the turgescient perineum.

Any insoluble nitrogen increment that may have occurred, might have been fibrous or cellular. The extra-cellular insoluble nitrogen of the perineum is located in the collagen and elastic fibres. In order to gain independent evidence of an increase in insoluble nitrogen, the mass of collagen in the perineum was determined at the two stages of the cycle. No estimation was made of any non-protein accretion to the collagen fibres. If the mucopolysaccharides bound to the collagen increased in mass, then an increase in the insoluble nitrogen mass would have resulted, but such an increase would not have been shown by this second estimation.

3. The collagen content of the perineum.

The mass of perineal collagen did not vary significantly during perineal expansion (Table 6). Insignificant variations of -1.5 g. and +3.1 g. were found. The apparent increase of 3.1 g. in B. 354 was probably due to the increased experimental error caused by the unusually large increase in perineal mass that was observed in this animal. The inference that the mass of collagen did not vary was supported by the virtual absence of hydroxyproline (less than 2 mg./100 ml.) in Fraction A (the tissue fluid), and in the blood plasma. Thus, no soluble, hydroxyproline-containing, collagen

TABLE 6.

The mass of hydroxyproline and collagen in the perinea of six baboons at maximum turgescence and at deturgescence.

Animal Number	Concentration of hydroxyproline. mg. per cent		Mass of hydroxyproline mg.		Increase in mass of hydroxyproline. mg. u - v	Mass of collagen. grams		Increase in mass of collagen. grams x - y
	Turg.	Deturg.	Turg. u	Deturg. v		Turg. x	Deturg. y	
B. 362	10	240	170	580	-210	1.2	2.7	-1.5
B. 316	20	280	320	400	- 80	2.3	2.9	-0.6
B. 319	30	250	530	610	- 80	3.8	4.3	-0.5
B. 344	80	400	1180	940	+240	8.4	6.7	+1.7
B. 253	50	240	840	460	+380	6.0	3.3	+2.7
B. 354	30	320	1330	890	+440	9.5	6.4	+3.1

precursors were detectable. The presence of a constant mass of collagen suggests that the apparent increase in insoluble nitrogen was not real. However, if there was a real increment of insoluble nitrogen, then it was presumably either cellular, mucopolysaccharides or phospholipids, all of which, in any case, would only contribute a small proportion to the total nitrogenous increase.

The mass of total nitrogen in the sex skin increased as the tissue became swollen. The mass of soluble nitrogen in the perineum

increased during the follicular phase of the ovarian cycle.

The increment in soluble nitrogen was presumably contributed to in varying proportions by proteins, mucopolysaccharides and phospholipids. The increase in soluble nitrogen was partitioned between the interstitial fluid and the formed elements of the sex skin tissue. The contribution of soluble proteins to the nitrogen increase and the partition of the soluble proteins between the tissue fluid and the formed elements was determined by estimating the variation in the mass of the protein of the tissue fluid during the menstrual cycle.

4. Tissue fractionation.

Perineal tissue fluid was obtained by gentle centrifugation of whole perineal tissue (Figure 4 (1)).

The tissue fluid (Fraction A) was a water-clear solution. No nuclear or cytoplasmic material was microscopically visible in this solution. The only solid material visible under the microscope was a few small refractile granules which were probably broken-off cell processes or cytoplasmic granules. Fraction A was presumably derived from the intercellular fluid of the tissue. Perineal tissue samples centrifuged at 1,500 r.p.m. at 2° C. for 30 minutes yielded perineal fluid containing less than 0.5 per cent blood plasma (Cohen, 1956). Perineal tissue was not very cellular, especially when it was swollen. Therefore, only small amounts of cell contents were

separated with Fraction A, which was therefore assumed to be identical with perineal tissue fluid. However, a certain unknown proportion of Fraction A can be expected to have adhered to the fibrous and cellular elements of the tissue. Furthermore, a certain unknown amount of the constituents of this solution may have been bound to solid tissue elements. Therefore, the mass of soluble proteins in the perineal tissue fluid was probably consistently underestimated.

Fraction A was not identical with the saline extract in which soluble nitrogen concentrations were estimated (compare Figures 4 (1) and 4 (2)). On the other hand, the discrepancy which arose from the presence of cellular and intra-vascular soluble nitrogen could not have been gross. Some measure of agreement was expected between the mass of soluble nitrogen estimated in the saline extract and the mass of nitrogen calculated from the protein estimated in the tissue fluid.

Fraction B was a white, dense, solid mass of connective tissue fibres and cells which presumably contained a certain proportion of Fraction A and some blood plasma, blood vessels, lymph, lymph vessels, nerves and blood cells. Fraction B contained 0.33 g. per cent hydroxyproline at turgidity and 0.39 g. per cent at perineal rest. The collagen content of Fraction B did not alter markedly during the menstrual cycle, but was constant at 2.4 to 2.8 g. per cent. This indicates that approximately the same proportion of fluid was left in Fraction B in all the samples.

The proportions of the two fractions varied during the menstrual cycle. Different perinea contained from 29 to 35 per cent of Fraction B at rest and from 3 to 27 per cent at maximum turgidity. Duplicate estimations in each case differed by less than 5 per cent.

5. The proteins of Fraction A (perineal tissue fluid).

Fraction A was a protein-containing solution (Table 7). The total protein concentration varied from 2.65 g. per cent (B. 362) to 4.44 g. per cent (B. 316) in different animals. No consistent variation was evident in the protein concentration during the ovarian cycle. The constant concentration of perineal fluid proteins implies a physiological regulation of perineal fluid proteins. In every animal the total protein concentration of Fraction A was above 2.5 g. per cent (Table 7). In different animals, albumin concentrations in perineal fluid of 1.34 g. per cent (B. 354) and 3.34 g. per cent (B. 316) were found. No consistent variation in the albumin concentration during the menstrual cycle was detected. The globulin concentration ranged from 0.56 g. per cent (B. 354) to 1.47 g. per cent (B. 344) and no consistent variation during the cycle was demonstrable. In all cases, the maximum globulin concentration was below 2.0 g. per cent. The albumin/globulin ratio of Fraction A was 0.95 and 4.73 at the two extremes of the cycle in B. 354.

TABLE 7.

Protein concentration (g. per cent)
in Fraction A (perineal tissue fluid)
in eight baboons at maximum turgescence and at deturgescence.

Animal Number	Total protein g. %		Albumin g. %		Globulin g. %		A/G	
	Turg.	Deturg.	Turg.	Deturg.	Turg.	Deturg.	Turg.	Deturg.
B. 316	4.44	3.73	3.34	2.30	1.10	1.43	3.04	1.61.
B. 316	4.25		2.92		1.33		2.20	
B. 319	3.94	3.98	2.64	2.99	1.30	0.99	2.03	3.02
B. 319	4.08		2.28		1.80		1.27	
B. 253	3.82	3.53	2.62	2.32	1.20	1.21	2.18	1.92
B. 344	3.50	2.90	2.03	1.72	1.47	1.18	1.28	1.38
B. 90	3.14		2.20		0.94		2.34	
B. 354	2.75	3.21	1.34	2.65	1.41	0.56	0.95	4.73
B. 354	3.33	3.42	2.40	2.81	0.93	0.61	2.58	4.61
B. 362	2.65		1.47		1.18		1.25	
B. 260		3.54		2.64		0.90		2.93

The volume of Fraction A was calculated from the proportions of the two fractions and the total volume of the perineum. The mass of protein in Fraction A at the two stages of the menstrual cycle was derived from the concentration of protein and the volume of Fraction A (Table 8).

The mass of protein in Fraction A (perineal tissue fluid) increased noticeably during the first part of the cycle. For example, in B. 316

TABLE 8.

Mass of protein in Fraction A (perineal tissue fluid) in six baboons at maximum turgescence and at deturgescence.

Animal Number	Volume of Fraction A. ml.		Protein concentration in Fraction A g. per cent		Mass of protein in Fraction A grams		Increase in mass of protein. grams x - y
	Turg.	Deturg.	Turg.	Deturg.	Turg. x	Deturg. y	
B. 344	1250	140	3.5	2.9	43.8	4.1	39.7
B. 316	1100	90	4.4	3.7	44.0	3.3	40.7
B. 253	1200	120	3.8	3.5	45.6	4.2	41.4
B. 319	1300	150	3.9	4.0	50.7	6.0	44.7
B. 354	3550	170	2.8	3.2	110.1	5.4	104.7
B. 362	1000	100	2.7		27.0		

the mass of perineal fluid protein rose from 3.3 g. to 44.0 g., while in B. 354 it increased from 5.4 g. to 110.1 g. The mass of protein in the perineal tissue fluid increased during the follicular phase of the ovarian cycle by 104.7 g. in B. 354, and by approximately 40 g. in four other animals. Thus, substantial increments were observed in the mass of protein in the perineal fluid as the sex skin expanded. An increase in the protein of the tissue fluid made a large contribution to the increase of soluble nitrogen in the whole tissue (Table 8).

The size of the contribution was estimated by calculating the increment of protein nitrogen in Fraction A from the protein increase in this fraction. The increase in protein nitrogen of the perineal tissue fluid was compared with the increase in total nitrogen in the whole tissue (Table 9).

A fairly close correspondence was evident between the increment in protein nitrogen in tissue fluid and the increment in total nitrogen in whole tissue. In all five animals examined, the increment in protein nitrogen was at least 70 per cent of the increment in total nitrogen. The increment in total nitrogen in the whole tissue was consistently greater than that in protein nitrogen in tissue fluid. The difference of 6.4 g. in B. 354 was possibly exaggerated by the large increase in perineal mass which this animal exhibited. The discrepancy of from 1 to 7 g. could have been due to three causes. Firstly, the presence of an increment in non-protein nitrogen in Fraction A would have given rise to such a discrepancy. Secondly, the estimation of protein

TABLE 9.

Increase in protein nitrogen in Fraction A (tissue fluid)
and in total nitrogen in whole perineal tissue during
perineal turgescence.

Animal Number	Protein nitrogen in Fraction A. grams x	Total nitrogen in whole tissue grams y	y - x grams
B. 316	6.5	7.7	1.2
B. 319	7.1	8.5	1.4
B. 344	6.4	7.8	1.4
B. 253	6.6	8.6	2.0
B. 354	16.8	23.2	6.4
B. 362		11.9	

in only Fraction A omits any increment in soluble nitrogen occurring in Fraction B, namely the formed elements. Thirdly, all of Fraction A was not separated by the gentle centrifugation. A proportion of Fraction A and its protein constituents was undoubtedly left in Fraction B.

The tissue fluid proteins increased by 40 g. and 105 g. as the sex skin became turgescient. The increase in tissue fluid proteins accounted for a large part of the increase in total tissue soluble nitrogen.

The increment in tissue fluid protein may have been due to plasma proteins or to organ-specific perineal proteins. The proteins of the perineal fluid were therefore compared with the baboon plasma proteins by electrophoresis and salt fractionation.

A visual examination of the stained electrophoretic patterns revealed that Fraction A contained proteins of similar mobility to the plasma proteins. In Fraction A (the tissue fluid) there were components analagous to gamma, beta, and alpha globulins and albumin. In addition, there was a component not unlike fibrinogen which remained at the origin. This component, however, appeared to have been precipitated because it did not radiate and diffuse as did the other fractions. The graphs of the electrophoretic patterns showed two high peaks analagous to albumin and fibrinogen, and other peaks corresponding in mobility to the gamma, beta, alpha-2 and alpha-1 globulins of the same animal's blood plasma (Figures 6 and 7).

The protein of the perineal fluid was fractionated into two portions by means of 28 per cent sodium sulphite, as was blood plasma. The concentrations of these so-called albumins and globulins are shown in Table 7.

The proteins of perineal tissue fluid and baboon blood plasma were similar in electrical behaviour and could both be fractionated by 28 per cent sodium sulphite. These two properties suggest a similarity between blood plasma proteins and perineal tissue fluid

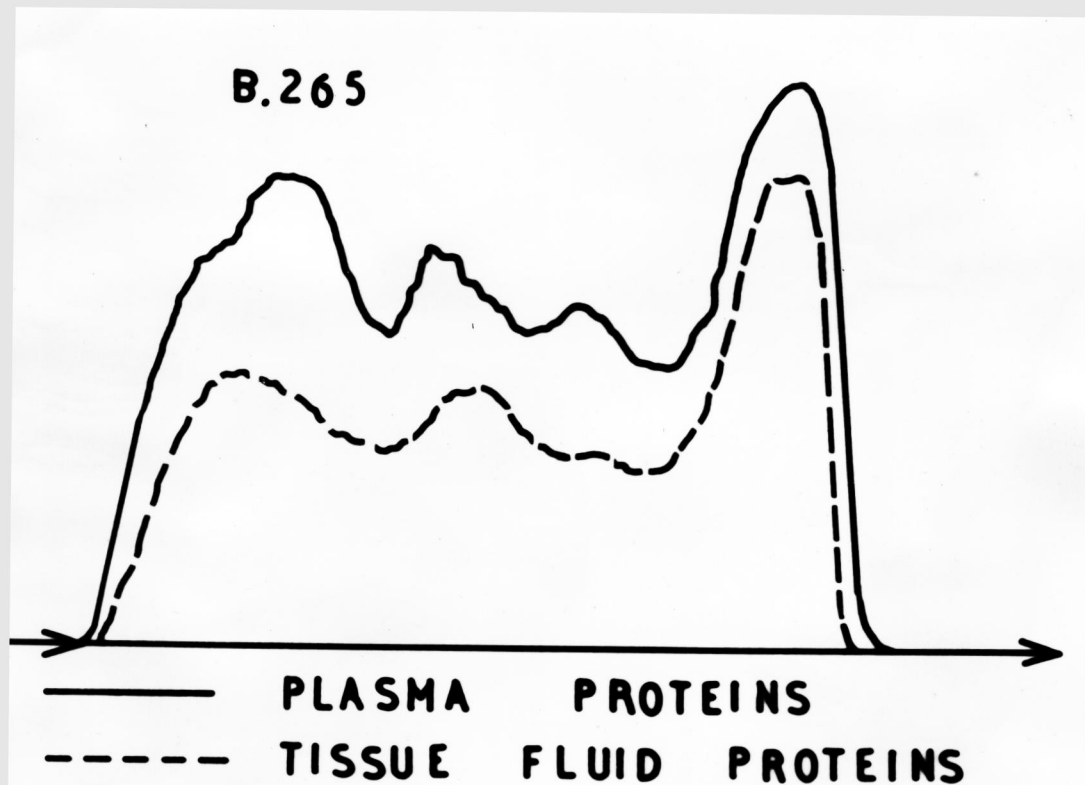


Figure 6. Electrophoretic pattern of plasma proteins (upper line) and tissue fluid proteins (lower line) in B. 265. Phosphate buffer, pH = 7.5, μ = 0.1.

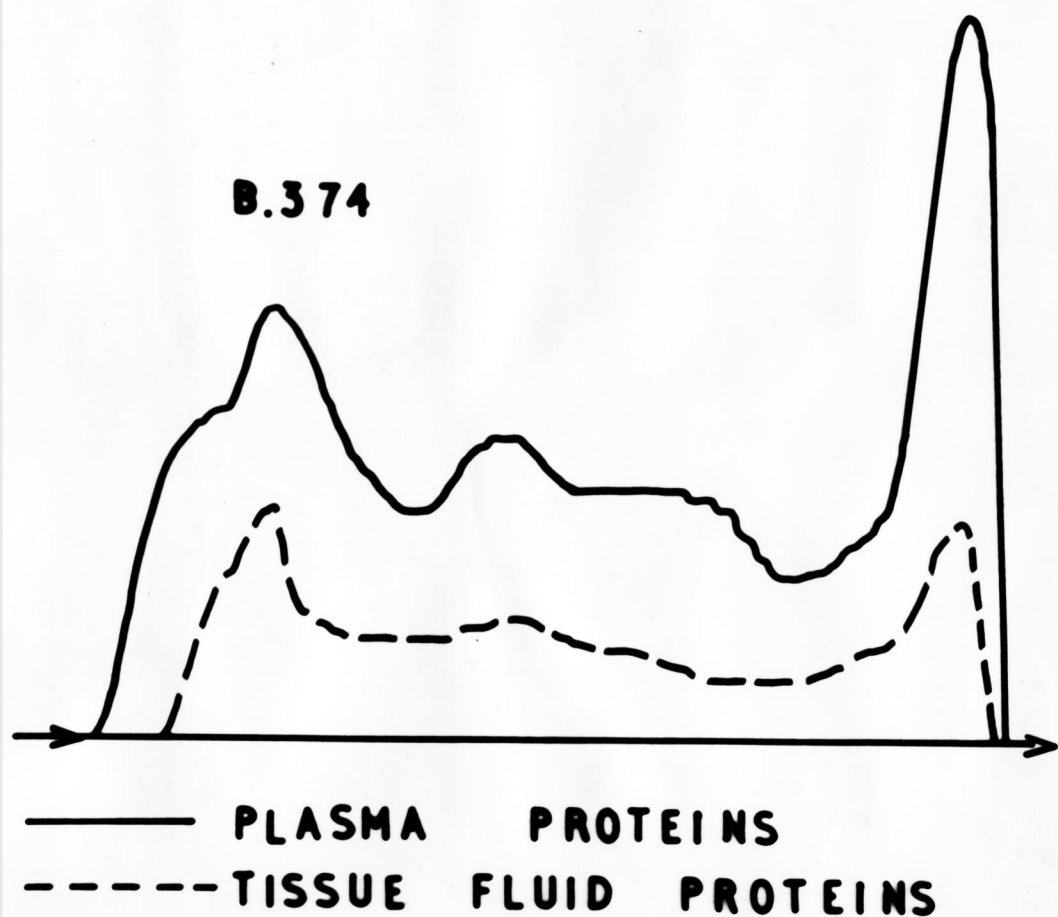


Figure 7. Electrophoretic pattern of plasma proteins (upper line) and tissue fluid proteins (lower line) in B. 374. Phosphate buffer, $\text{ph} = 7.5$, $\mu = 0.1$.

proteins, but do not prove their identity. Additional evidence for their identity lies in the decrease of the mass of circulating plasma proteins during the follicular phase of the cycle and in an abrupt increase in the mass of circulating plasma proteins which accompanies deturgescence (Cohen, 1954 b). Further, electrophoretic and ultra-centrifugal analyses and studies with radio-iodine suggest that the protein in the perineal tissue fluid consists of extra-vascular plasma protein (Cohen, 1956). The rigorous identification of the perineal tissue fluid proteins and blood plasma proteins requires a similarity in the ultra-centrifuge, in the Tiselius electrophoresis apparatus, in reactions to salts, in amino acid composition, in serological properties and in other respects. In short, in order to identify two proteins with certainty, every known property of the proteins should be shown to be identical. The organic chemist's methods of identification are inadequate because of the complexity of the proteins and because of our ignorance of the true constitution and properties of proteins.

From the evidence presented above it was deduced that the baboon plasma proteins contributed to the increase in perineal tissue proteins.

Additional information was sought by comparing the concentrations of plasma and tissue fluid proteins.

The total protein concentration of the blood plasma varied from 6.12 g. per cent (B. 354) to 10.21 g. per cent (B. 354) (Table 10).

TABLE 10.

Protein concentration (g. per cent) in the blood plasma of eight baboons at maximum turgescence and at deturgescence.

Animal Number	Total protein g. %		Albumin g. %		Globulin g. %		A/G	
	Turg.	Deturg.	Turg.	Deturg.	Turg.	Deturg.	Turg.	Deturg.
B. 319	6.75	6.68	3.48	4.24	3.27	2.44	1.06	1.74
B. 316	7.47	7.36	3.97	4.16	3.50	3.20	1.13	1.30
B. 362	7.25		4.07		3.18		1.28	
B. 354	6.12	10.21	2.39	5.87	3.73	4.34	0.64	1.35
B. 344	7.08	8.18	3.65	3.72	3.43	4.46	1.06	0.83
B. 90	9.06	6.65	3.49	1.65	5.57	5.00	0.63	0.33
B. 260		11.25		7.14		4.11		1.74
B. 253	6.42		3.70		2.72		1.36	

The concentration varied according to the stage of the menstrual cycle at which the sample was taken (Cohen, 1954 b). The total protein concentration did not fall below 6.0 g. per cent. The albumin concentration varied from 1.65 g. per cent (B. 90) to 7.14 g. per cent (B. 260) and the globulin concentration from 2.44 g. per cent (B. 319) to 5.57 g. per cent (B. 90). A/G ratios of 0.33 (B. 90) and 1.74 (B. 319, B. 260) were observed. Electrophoretic patterns demonstrated albumin, alpha, beta, and gamma globulin and

TABLE 11.

Ratio of the total protein concentration in blood plasma
to the protein concentration in Fraction A (tissue fluid)
in five baboons at maximum turgescence and at deturgescence.

Animal Number	Turg.	Deturg.
B. 316	1.68	2.69
B. 319	1.71	1.68
B. 344	1.75	2.82
B. 354	2.22	3.18
B. 362	2.73	

fibrinogen components. The proportions of these several components and their variation during the menstrual cycle were similar to those described by Cohen (1954 b).

The ratio of total protein in blood plasma and in Fraction A varied from 1.68 (B. 316) to 3.18 (B. 354) (Table 11). The concentration of total protein in the blood plasma was at least 60 per cent greater than the protein concentration of perineal fluid. The concentration of rabbit plasma proteins is about 3.2 times that of the protein in the interstitial tissue of rabbit muscle (Gitlin and Janeway, 1954). The protein concentration of baboon blood plasma was, on the average,

TABLE 12.

Ratio of albumin/globulin in Fraction A (tissue fluid)
to albumin/globulin in blood plasma in five baboons
at maximum turgescence and at deturgescence.

Animal Number	Turg.	Deturg.
B. 362	0.98	
B. 344	1.21	1.75
B. 354	1.48	3.50
B. 319	1.93	1.74
B. 316	2.69	1.27

2.3 times greater than the protein concentration of Fraction A.

The ratio between the Fraction A A/G and the blood plasma A/G was above unity in 8 of the 9 examinations (Table 12). In other words, the proportion of albumin in the tissue fluid was greater than in the blood plasma. The proportion of electrophoretic albumin and the more slowly sedimenting ultra-centrifugal component is somewhat lower in blood plasma than in perineal tissue fluid (Cohen, 1956). These facts suggest that proportionately more albumin passes in and out of the perineum than does globulin; that is, albumin diffuses more rapidly.

While fractionating the perineal tissue, a gel of varying consistency was found in the tissue fluid. The gel was found every time perineal tissue was centrifuged at room temperature. The gel did not appear if the tissue was allowed to stand at room temperature for more than 15 minutes before centrifuging.

When the tissue was centrifuged at 2° C. a gel was not found in the fluid, but the fluid gelled after 24 hours at 2° C. or within a few minutes at room temperature.

The addition of 0.1 mg. heparin to 5 ml. of fluid separated in the cold prevented gel formation, even when the fluid was subsequently warmed to room temperature. Similarly, 2 mg. ammonium oxalate in 5 ml. fluid separated in the cold prevented gelation completely. 50 units of thrombin in 2 ml. of fluid separated in the cold gave rise to a gel within 2 minutes at 2° C. Neither heparin nor oxalate prevented the formation of a gel when the tissue fluid was separated at room temperature.

On mechanical agitation of about 5 ml of gel, a fluid was expressed and the gel was resolved into several milligrams of insoluble white fibres. A sample of these fibres was hydrolyzed in 2 normal sulphuric acid for 20 hours and then neutralized with solid barium carbonate. The solution was centrifuged and the supernatant was spotted on filter paper. The spot did not stain with p-anisidine hydrochloride which colours reducing sugars (Block, Durrum and Zweig, 1956). A ninhydrin

reagent gave a violet colour with the spot. This means that the fibrous material contained no reducing sugars, but did contain amino acids. The fibrous material contained no detectable hydroxyproline and was therefore not collagen.

The gel itself was soluble in 2 Molar urea, but the fibrous material was not soluble in this solution.

The gel resembled a fibrin gel in the following respects. Heparin and oxalate prevented, while thrombin accelerated the formation of the gel. The gel was quite soluble in 2 Molar urea, but the solid fibrous material was not soluble in 2 Molar urea. The fibrous material contained no reducing sugars and although amino acids were present, the fibres were not collagen.

6. Increment in non-protein material.

The increase in the protein mass of Fraction A (tissue fluid) accounted for about 40 g. to 110 g. of the total increase in perineal mass. In addition to the increase in protein, other solid constituents of the sex skin contributed to the total tissue increase.

The dry material contributed from about 60 g. (B. 316) to about 150 g. (B. 354) to the total increase in perineal mass in different animals (Table 13). The dry material increased 3 (B. 319) and 7 (B. 316) times from the resting to the maximum level in two animals.

TABLE 13.

Dry weight of perineal tissue in six baboons
at maximum turgescence and at deturgescence.

Animal Number	Percentage dry weight		Mass of dry material.		Ratio of Turg./Deturg. dry mass grams $\frac{x}{y}$	Increment in dry mass grams x - y
	g. per cent. Turg.	g. per cent. Deturg.	grams Turg. x	grams Deturg. y		
B. 316	4.40	7.17	69.9	10.3	6.8	59.6
B. 319	5.58	14.93	108.9	36.2	3.3	72.7
B. 344	6.62	11.60	102.0	27.4	3.7	74.6
B. 253	5.44	7.52	91.8	14.1	6.5	77.7
B. 362	7.74	9.98	95.2	15.9	6.0	79.3
B. 354	4.46	11.68	180.0	32.1	5.6	147.9

In all six baboons the percentage of dry material was significantly higher during perineal rest than during turgescence. In the turgid state the percentage of dry weight varied from 4.40 g. per cent (B. 316) to 7.74 g. per cent (B. 362) and in the flaccid state from 7.17 g. per cent (B. 316) to 14.93 g. per cent (B. 319). The perineal tissue underwent a varying degree of dilution as it progressed from the resting to the expanded state.

The largest portion of the perineal increase was contributed by an influx of water (Table 14). 1 Kg. and 3.6 Kg. of water entered the perinea of B. 362 and B. 354 respectively, during the follicular phase of the ovarian cycle. The mass of water in the resting perineum increased 7 (B. 344) and 16 (B. 354) times during turgescence in two of the animals examined.

TABLE 14.

Mass of water in the perinea of six baboons
at maximum turgescence and at deturgescence.

Animal Number	Mass of water grams		Ratio of Turg./Deturg. mass of water $\frac{x}{y}$	Increase in mass of water grams $x - y$
	Turg. x	Deturg. y		
B. 362	1140	140	7.9	1000
B. 344	1440	210	6.9	1230
B. 253	1600	170	9.2	1430
B. 316	1520	130	11.4	1490
B. 319	1840	210	8.9	1630
B. 354	3860	240	15.9	3620

The increment in total body weight is compared with the increment in perineal mass in Table 15. The increase in perineal mass in all five cases was apparently greater than the corresponding increase in body weight.

The body weight was estimated to the nearest 100 g. and the volume of the turgid perineum with an accuracy of ± 100 ml. Therefore, a discrepancy of 0.4 Kg. in body weight and in perineal mass was not regarded as significant. The measurement of perineal mass was possibly consistently overestimated due to irregularities of the perineum and to the unknown depth of the reactive tissue. Occasionally, although the perineum expands normally, the body weight increases only slightly, due to a dissociation between body weight increase and perineal expansion (Gillman and Gilbert, 1956 b). In three successive cycles, three months prior to the present investigation, B. 316 showed body weight increases of 2 Kg., 0.0 Kg. and 2 Kg. respectively (Figure 8). The absence of the normal body weight increase accompanied by a normal perineal expansion can only be detected by measuring several successive cycles.

The data presented in Table 15 suggest that the perineum receives increments from other body tissues, but more precise experimental methods are required to establish definitely the redistribution of body tissues to the perineum.

At the present time it can only be asserted that the bulk of the increase in perineal mass was due to material derived from the diet.

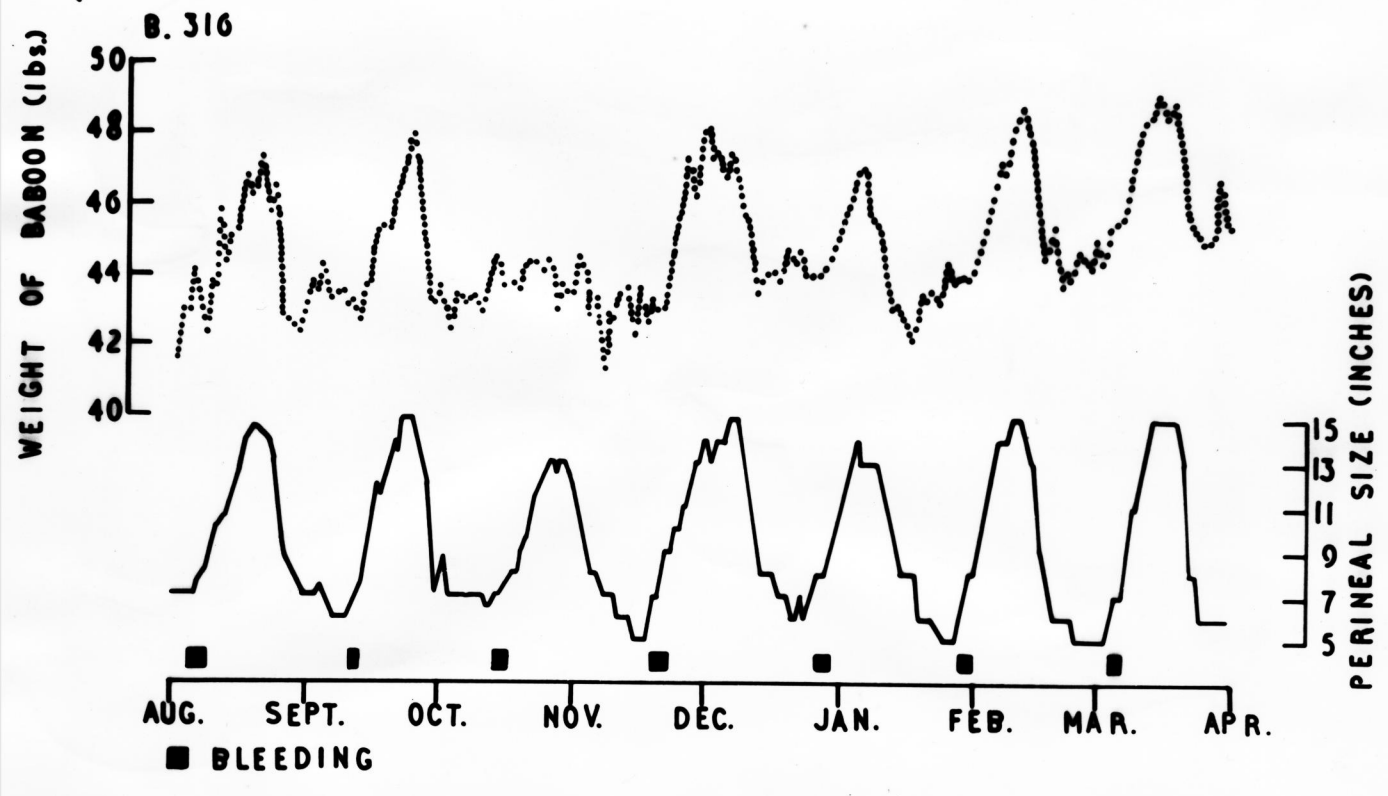


Figure 8. Variations in body weight and perineal size during the menstrual cycle in B. 316.

TABLE 15

Weight of five baboons at the stage of
maximum turgescence and at deturgescence.

Animal Number	Weight of baboon		Increment in weight of baboon Kg. x	Increment in perineal mass Kg. y	y - x Kg.
	Turg.	Deturg.			
B. 319	19.9	18.5	1.4	1.7	0.3
B. 344	16.7	15.7	1.0	1.3	0.3
B. 253	16.6	15.6	1.0	1.5	0.5
B. 362	11.9	11.4	0.5	1.1	0.6
B. 316	21.0	20.3	0.7	1.4	0.7

A few preliminary comparisons were made of the sodium, potassium and chloride concentrations in the blood plasma and in the perineal tissue fluid of several baboons. The tissue fluid was obtained by centrifuging the whole tissue at 2° C (Figure 4 (1)). The sodium and potassium estimations were performed with a Beckman Model B flame photometer. The chlorides were estimated by titration with mercuric nitrate using S-diphenyl-carbazone as indicator (Schales and Schales, 1941; Asper, Schales and Schales, 1947). All three electrolytes were estimated with an accuracy of ± 2 per cent. The results are shown

in Tables 16 and 17.

Even these few results indicate that the concentration of electrolytes in the tissue fluid does not passively follow the corresponding concentration in the blood plasma. Consequently, it is possible that a specific mechanism exists which is responsible for the passage of electrolytes across the capillary endothelium. However, a full

TABLE 16

Concentration (milli-equivalents per litre) of sodium and potassium in blood plasma and perineal tissue fluid in seven baboons during perineal turgescence.

Animal Number	Blood	Sodium	Blood Tissue	Blood	Potassium	Blood Tissue
	m. eq./l. x	m. eq./l. y		m. eq./l. u	m. eq./l. v	
			$\frac{x}{y}$			$\frac{u}{v}$
B. 260	159	150	1.06	4.5	5.6	0.80
B. 260	145	139	1.04	3.4	5.5	0.62
B. 295	157	150	1.05	4.2	5.2	0.81
B. 371	150	134	1.12	4.7	4.9	0.96
B. 265	173	131	1.32	4.7	7.8	0.60
B. 286	150	126	1.19	5.2	6.2	0.84
B. 396	138			3.5	4.8	0.73
B. 365	138			4.6		

TABLE 17.

Concentration (m. eq./l.) of chloride
in blood plasma and perineal tissue fluid
in four baboons during perineal turgescence.

Animal Number	Blood m. eq./l. x	<u>Chloride</u> <u>Tissue</u> m. eq./l. y	<u>Blood</u> <u>Tissue</u> $\frac{x}{y}$
B. 260	112	115	0.97
B. 295	116	116	1.00
B. 371	122	117	1.04
B. 265	117	106	1.11

investigation into the electrolytes of the tissue fluid is required before any conclusions can be drawn concerning the regulation of tissue fluid electrolytes by either the blood plasma or the capillary endothelium.

SECTION D

DISCUSSION

1. Introduction.

The experiments performed in this study (see Section C) demonstrated that the mass of the sex skin of the Chacma baboon increased by about 1 to 4 Kg. as the sex skin progressed from the resting to the expanded state. The major portion of the increase in mass was due to an influx of water. Nitrogenous substances contributed substantially (7.7 to 23.2 g. nitrogen) to the increase in mass of solid material that occurred during the follicular phase of the ovarian cycle. Increases of 6 to 15 g. of soluble nitrogen were accompanied by small or no increases in insoluble nitrogen and by no increases in the mass of the collagen of the tissue. The perineal tissue fluid proteins increased by approximately 40 g. in each of four animals and by 105 g. in a fifth animal. The proteins in the tissue fluid possessed similar electrophoretic mobilities to the plasma proteins, and were present in about half the concentration of the plasma proteins.

In general terms, the cyclic expansion and contraction of the sex skin may be divided into three phases. Firstly, there is the entry of the solution into the perineum. Secondly, this fluid is retained in the perineum. Thirdly, the fluid leaves the perineum.

In addition, it is necessary to consider whether the proteins are altered in any way as they pass through the perineum, and whether there is any protein synthesis, degradation or amino acid incorporation of the shifting proteins.

The cycle may also be considered as the resultant of the two apposing movements in and out of the perineum. This latter formulation takes into consideration the kinetics of the various movements.

The explanation of the phenomenon may be arbitrarily divided into a vascular and a tissue component. The latter in turn can be subdivided into water, electrolyte and nitrogenous components.

2. Nitrogen movements.

The absolute total nitrogen mass in the sex skin of all six baboons increased markedly as the perineum became turgid. The increments in total nitrogen mass varied from 7.7 g. (B. 316) to 23.2 g. (B. 354). The mass of soluble nitrogen in the perinea of the six baboons also increased during the follicular phase. The mass of insoluble nitrogen increased very slightly or not at all. The protein fraction of the tissue collagen did not apparently contribute to the nitrogen increment.

The increment in soluble nitrogen was due mainly to an increase in the soluble proteins of the tissue fluid, which increased by about 40 g. in each of four animals and by 105 g. in one animal. Cohen (1955, 1956) demonstrated that between 50 g. and 60 g. of protein are deposited in the sex skin during the follicular phase of the

cycle, and that roughly 30 g. of protein leave the perineum within the first 2 to 4 days of deturgescence.

Fraction A (tissue fluid) accounted for the majority of the increase in the tissue mass and in the mass of soluble protein. The total protein concentration of Fraction A varied between 2.7 g. per cent (B. 362) and 4.4 g. per cent (B. 316). Salt fractionation in 28 per cent sodium sulphite separated baboon blood plasma into albumin and globulin with a fair degree of electrophoretic homogeneity (Anderson and Cohen, 1951; Cohen, 1954 b). This method also separated the proteins of Fraction A into two moieties which were presumably albumin and globulin. Paper electrophoresis of Fraction A showed the presence of components with similar electrophoretic mobilities to the albumin and globulins of baboon blood plasma (Figures 6 and 7).

At maximum turgidity the perineal tissue shows grossly expanded extracellular spaces and swollen cells. In the monkey the perineal swelling consists essentially of oedematous connective tissue, and during turgescence there occurs a dispersion of cell nuclei indicative of a considerable accumulation of intercellular fluid (Zuckerman and Parkes, 1932).

These several observations suggest that the increase in perineal protein is mainly due to an increase of extra-vascular plasma proteins situated primarily in the intercellular spaces of the perineal tissue.

(a) Source of new perineal protein.

There are three possible sources of this new protein that is lodged in the perineum during turgescence.

All the new protein may be formed de novo in the perineum. This explanation postulates a constant extra-perineal plasma protein pool size, and a cyclic expansion and contraction of the perineal protein mass. Alternatively, the source of the new protein may be the body plasma protein pool; blood, lymph, extravascular and possibly intracellular. In the latter case, there would be a constant plasma protein pool size and a cyclic alteration of the proportions in the intra-vascular, extravascular and perineal compartments. Thirdly, increased synthesis of plasma proteins during turgescence may account for the new protein. This latter possibility implies a regular increase and decrease of the total plasma protein pool size.

(b) De novo synthesis in the perineum.

The data presented in Section C militate against the de novo synthesis of the new protein in the perineum. The new protein in the perineum appeared to be plasma protein, containing both albumin and globulins. The fluid expressed from the swollen sexual skin of the rhesus monkey contains three ultracentrifugal components, two of which resembled normal serum proteins (Ogston, Philpot and Zuckerman, 1939). The proteins in baboon perineal tissue

fluid are ultracentrifugally and electrophoretically similar to blood plasma (Cohen, 1956). Proteins similar in nature to those present in blood serum are found in the ground substance of connective tissue (Ropes and Bauer, 1953).

It is doubtful whether connective tissue is capable of synthesising all the varieties of plasma proteins. However, the participation of the perineum of the Chacma baboon in plasma protein synthesis has been suggested (Cohen, 1954 a). No amino acid incorporation into plasma albumin occurs in the carcass of a hepatectomised animal (Miller, Bly and Bale, 1954). That is, connective tissue is unable to synthesise plasma albumin. Therefore, the perineal tissue was probably not capable of synthesising the albumins which accumulated there in the follicular phase of the ovarian cycle. In addition, there was a simultaneous loss of all plasma proteins from the blood as the perineum enlarged, and an increase with perineal involution, suggesting a passage of protein between the blood plasma and perineum (Cohen, 1955). However, the connective tissues of the sex skin may synthesise globulins in common with other connective tissues and at a similar rate. Such a synthesis may be linked with the sexual expansion of the perineum.

(c) Extra-perineal source of the new protein.

The sex skin is apparently not the sole and specific source of the new protein. The information provided by the present investigation does not enable one to decide whether there is an increased protein synthesis or merely a redistribution of preformed protein. The decrease in intravascular plasma protein mass during the follicular phase of the ovarian cycle is insufficient to account for the increase in perineal protein (Cohen, 1955). This indicates that there is either a synthesis of new plasma protein during turgescence or that there is a redistribution of preformed extravascular protein. The extravascular protein may be intracellular or in the tissue fluid.

In order to differentiate these two possibilities two facts are of paramount importance. They are the total size of the plasma protein pool and the rate of plasma protein synthesis during the menstrual cycle. Provided with these data it might be possible to establish whether the new protein is synthesised by increasing the rate of plasma protein synthesis and thereby increasing the total plasma protein pool, or whether it is obtained by a redistribution of preformed protein. In the latter case there would be no increase in the rate of protein synthesis or in the plasma protein pool during the follicular phase of the cycle.

(d) The size of the plasma protein pool.

The total exchangeable plasma protein pool increased during turgescence and contracted during deturgescence (Cohen, 1956). The proportion of labelled protein retained in the circulation 100 hours after injection was 33 per cent during perineal resorption and only 18 per cent during turgescence. The urinary excretion of radio-iodine, on the other hand, was constant during both phases of the cycle (22.4 to 25.4 per cent of the dose after 100 hours). These facts suggest that there is a larger plasma protein pool in the follicular phase of the cycle (Cohen, 1956). The size of the pool was estimated to be 128 g. and 130 g. during perineal resorption and 150 g. and 151 g. on the 8 - 9th day of perineal hypertrophy in two different animals respectively.

Additional support for an increased pool size was derived from the rate of excretion of radio-iodine in the urine; and the rate of plasma protein catabolism calculated therefrom. In the second half of the menstrual cycle the rate of catabolism rose above the level observed during the latter two weeks of turgescence.

Further, the rate of plasma protein synthesis calculated from the apparent half-life of total plasma protein ($0.693/t_{1/2}^{\text{app}}(\text{days} \times 100)$) was 5.1 per cent of the pool per day during perineal turgescence. Between the 5th and 12th days of the luteal phase the decay of plasma radio-activity ceased and total protein specific activity varied

by less than one per cent in five successive estimations. These results suggest that the rate of plasma protein synthesis is considerably or even completely inhibited during part of the luteal phase.

The net result of these rates of synthesis and catabolism was that the total plasma protein pool size increased progressively from 117 g. at the onset of menstrual bleeding to 192 g. at the end of perineal turgescence. Between the 5th and 12th days of the luteal phase the pool size diminished progressively from 195 g. to 154 g.

However, the validity of data obtained by the use of iodinated protein has been seriously questioned in recent years (Berson, Yalow, Schreiber and Post, 1953; Armstrong, Kukral, Heishman, McLeod, Wolter and Bronsky, 1955; Masouredis and Beeckmans, 1955; Volwiler, Goldsworthy, Macmartin, Wood, Mackay and Fremont-Smith, 1955). Other investigations have shown that plasma protein iodinated according to the method described by McFarlane (1956), which Cohen (1956) employed, provides data concerning the distribution and elimination of albumin and globulin closely similar to that obtained with the corresponding C^{14} -labelled fractions in both the rabbit (Cohen, Holloway, Matthews and McFarlane, 1956) and the rat (Cohen, 1956). Moreover, since I^{131} liberated by catabolism is promptly excreted

(Dixon, Bukantz and Dammin, 1951; Cohen, Holloway, Matthews and McFarlane, 1956), the rate of plasma protein catabolism and the pool size can be estimated repeatedly. These are precisely the events which it is desired to measure in the female baboon.

In the particular case of the female baboon there is one further, special objection to the radio-iodine labelling method. Plasma proteins may be segregated and stored in cells during the luteal phase of the cycle. These cells may be capable of removing the iodine as they absorb the plasma protein. Such a process will simulate plasma protein catabolism. The plasma proteins re-entering the "exchangeable" pool in the next follicular phase will simulate plasma protein synthesis. There is no positive evidence excluding this suggestion. Indirect evidence indicates that cells do not remove the iodine since radio-iodine bound to proteins has been detected inside cells (Crampton and Haurowitz, 1950; Haurowitz and Crampton, 1951). Nonetheless, definitive evidence is necessary before a decision can be reached on the reliability of radio-iodine data.

In view of the objections raised other evidence relating to a possible increase in plasma protein pool size was considered. The evidence shows that sites of plasma protein synthesis are available and also that the female baboon is probably able to manufacture plasma protein at an adequate rate.

Immune plasma globulin is formed in plasma cells (Fagreau, 1948). Chicken liver slices incorporate C^{14} -amino acids into plasma albumin (Peters and Anfinsen, 1950 a, 1950 b; Miller, Bly, Watson and Bale, 1951; Miller and Bale, 1954). The liver of fasting rats incorporates C^{14} -epsilon-lysine in all of the plasma proteins except gamma-globulin. A perfused eviscerated carcass incorporates radio-activity into the gamma-globulins (Miller, Bale, Yuille, Masters, Tishkoff and Whipple, 1949; Miller, Bly and Bale, 1954). By analogy, the liver and carcass of the female baboon is probably capable of synthesising the entire range of baboon plasma proteins.

Chicken liver can synthesise plasma albumin at the rate of 0.7 mg. albumin per g. liver protein per hour (Peters and Anfinsen, 1950 b). The liver of an adult female baboon weighs approximately 350 g. Assuming a protein content of 15 per cent; then the liver contains about 50 g. of protein. The mass of the exchangeable albumin in the protein pool of the female baboon increases by about 1 g. per day during turgescence (Cohen, 1956) representing a synthesis of 0.8 mg. albumin per g. liver protein per hour. Such a rate of synthesis is not far in excess of that found in chicken liver.

Rat liver can synthesise about 4.2 mg. plasma protein per g. liver protein per hour (Miller, Bly, Watson and Bale, 1951; Miller, and Bale, 1954). Intact rats synthesise plasma protein at the rate of 4.0 mg. per g. liver protein per hour (Abdou and Tarver, 1951).

About 4 g. of plasma protein accumulate in the turgescient perineum per day (Cohen, 1956). If all this protein is newly synthesised, it represents synthesis at the rate of 3.3 mg. plasma protein per g. liver protein per hour. The rate of plasma protein synthesis in the female baboon during perineal turgescence would be, at most only double the rate in the rat.

The female baboon is therefore probably able to expand her plasma protein pool during perineal turgescence and to contract it again during perineal involution.

(e) The distribution of plasma protein.

Absolute retention of plasma proteins within the blood vascular system does not occur. Thus plasma proteins are found in the lymph channels. Further, approximately fifty per cent of small (i.e. relative to plasma content) infusions of serum albumin disappear from the circulation within the first day (Fine and Seligman, 1944; Sterling, 1951). A considerable portion of the "lost" protein may be contained in the intercellular spaces. The dynamic equilibrium between intra-vascular and extra-vascular protein has been demonstrated with homologous antibody (Gitlin and Janeway, 1953). The volume of the extra-vascular space is considered to be about two or three times the plasma volume (Gamble and Robertson, 1952; Guadino, Schwartz and Levitt, 1948). The protein content of this extravascular fluid probably varies widely, the average

value lying somewhere between zero and 2 to 4 per cent (Drinker and Yoffey, 1941).

The total protein concentration of perineal tissue fluid is on the average 3.4 g. per cent, range 2.7 to 4.4 g. per cent (Shall, 1955) (Table 7). The protein content of sexual skin tissue fluid in three immature rhesus monkeys averaged 2.58 g. per cent (Fisher and Zuckerman, 1938). Cohen (1956) found that the total protein concentration of three samples of baboon perineal fluid obtained at intervals during turgescence was 2.0 to 2.3 g. per cent. The protein content of normal mammalian lymph is approximately 3.0 g. per cent, with a range from 0.5 to 4.0 g. per cent (Drinker and Field, 1933). There appears to be no significant difference between the protein content of baboon perineal tissue fluid and normal mammalian lymph. Normal lymph and tissue fluid contain protein in about equal concentrations (Drinker and Field, 1933). On this basis perineal tissue fluid and normal tissue fluids elsewhere in the body possess the same concentration of protein. The level of rabbit plasma proteins is about 3.2 times that of the protein in the interstitial tissue of rabbit muscle (Gitlin and Janeway, 1954). The concentration of protein in the blood plasma of most mammals fluctuates around 7.0 g. per cent. From this it follows that the protein concentration in rabbit interstitial connective tissue is about 2.2 g. per cent.

Taking into account the different techniques used, this value of 2.2 g. per cent is fairly close to the 3.4 g. per cent (Shall, 1955) and the 2.0 to 2.3 g. per cent (Cohen, 1956) found in baboon sex skin.

The extra-vascular plasma protein pool can therefore be expected to be equal to or larger than the intra-vascular pool. Thus there would be sufficient plasma protein to contribute a certain amount to the expanding perineum without an excessive drop in the concentration.

Another possible reservoir of plasma proteins lies within cells. Immunochemical studies have demonstrated the presence of heterologous proteins and homologous plasma proteins within cells (Coons and Kaplan, 1950; Coons, Leduc and Kaplan, 1951). Furthermore, that this represents a true penetration of gamma-globulin was indicated by injecting it into a child who was genetically unable to synthesise this protein. The child's tissues, which did not stain for gamma-globulin before injection, showed a normal staining pattern after injection (Gitlin, Landing and Whipple, 1953).

It is not known whether plasma proteins can reversibly enter and again leave the cell, although heterologous proteins have been shown to disappear from cells at about the same time as disappearance from the circulation should occur (Coons, Leduc and Kaplan, 1951). A quantitative approach to these experiments has not been possible,

so that the magnitude of the effects is unknown. However, it was noted that only traces of plasma proteins could be detected within muscle cells (Gitlin, Landing and Whipple, 1953). Therefore, any large cellular deposits would be found in the body cavities or in the connective tissues of the body. The evidence does indicate, however, that an intracellular accumulation of plasma proteins is a distinct possibility. In relation to the source of the baboon perineal plasma protein, we should bear in mind the possibility of an intracellular store of plasma protein which may be alternately discharged and resorbed.

The female baboon thus experiences a regular and recurrent physiological process immediately under the control of the sex hormones and in this respect differs from the male and from castrated females. Anything which interferes with the disposal of proteins, complex lipids, carbohydrates and electrolytes from the sex swelling in the post-ovulatory phase of the cycle may have far-reaching repercussions on the physiology of the organism.

Female baboons experience the disease known as primary amyloidosis, whereas castrate females and male baboons are free from this disease (Gillman and Gilbert, 1954).

The materials dislodged into the blood stream from the perineal swelling were derived from the intercellular matrix and were comprised mainly of water, electrolytes and plasma proteins. Preliminary

examination of the blood of a baboon which had developed primary amyloidosis revealed a very marked increase in the sedimentation rate and a profound change, amongst other things, in the plasma proteins. The globulins and fibrinogen were elevated and, electrophoretically, the globulin complex was fragmented into at least 10 small components; the albumins were abnormal and an unknown fraction migrated well ahead of the albumin (Shall, 1954).

The view has been expressed that in chronic suppuration a soluble protein circulates in the blood and under certain conditions is deposited in the tissues. The suggestion has been put forward that amyloidosis was due to chronic protein intoxication. Finally, it was believed that the development of amyloidosis was dependent on a hyperglobulinaemia (Gillman and Gilbert, 1954). All three views are compatible with an explanation involving the specific events which occur in the female baboon during the reproductive period of its life.

Summing up all the evidence available the likeliest explanation of the origin of the plasma protein in the perineum is that during the follicular phase there is increased plasma protein synthesis, decreased breakdown, and a redistribution of plasma proteins from the extravascular and intravascular compartments to the intercellular spaces of the perineum.

(f) The metabolism of plasma proteins in the perineum.

No experiments were performed which could give precise information as to the nature and extent of any alteration or metabolism of the plasma proteins in the sex skin. There is, in fact, no positive evidence that the proteins undergo any alteration or metabolism in the sex skin itself. Since the proteins in Fraction A showed similar electrophoretic mobilities to those in the blood plasma and no new proteins were apparent in Fraction A, it is likely that the proteins did not undergo any severe changes in passing through the capillary membrane. A clear answer to this problem requires the use of labelling techniques, the most useful of which at present are isotopes and antibodies.

(g) The A/G ratio in blood plasma and perineal tissue fluid.

The A/G ratio in Fraction A was consistently greater than the A/G ratio in the blood plasma (Table 12). The proportion of electrophoretic albumin and the more slowly sedimenting ultra ultracentrifugal component was higher in baboon perineal tissue fluid than in blood plasma (Cohen, 1956). The specific activity of plasma albumin and perineal fluid albumin was the same; On the other hand, all the globulin components in the perineum possessed a higher specific activity than did those in the blood plasma (Cohen, 1956). These findings may be explained by a binding of the globulins to something in the perineum or by a more rapid diffusion of the albumin across the capillary endothelium. If albumin diffuses more rapidly than globulins through the capillaries of the perineum, then there would

be a higher A/G ratio in the tissue fluid than in the blood plasma. Also the specific activity of the globulins in the perineum would be higher than in the blood plasma. In addition, the outpouring of albumin following ovulation would not result in a large increase in the plasma concentration, because it could be quickly distributed. The opposite would occur with the globulins. In fact, after ovulation the concentration of plasma globulins rises sharply, while the concentration of plasma albumins rises only slightly (Cohen, 1954 b).

(h) Initiation of the protein changes.

Oestrogen is known to initiate the perineal hypertrophy, with the consequent plasma protein changes. Cohen (1955) removed the sex skin from a castrate female baboon. Courses of oestrogen therapy repeated 3 and 10 weeks after excision of the perineum led to a reduction in plasma protein mass of only 3.7 g. and 1.1 g. respectively as against an average of about 6 g. in a normal animal and 6.0 g. and 14.8 g. in spayed animals on oestrogen therapy. The decrease in plasma protein concentration induced by oestrogen therapy in the castrate female baboon depends upon the occurrence of perineal swelling. Presumably, the same applies in the normal animal. The protein changes are probably dependent on hypertrophy and involution of the perineum and not directly on the presence of oestrogen. Hypoproteinaemia in a healthy animal is a powerful stimulant to protein synthesis (Madden and Whipple, 1940; Whipple, 1942;

1948; Whipple and Madden, 1944). As protein is transported into the sex skin a relative hypoproteinaemia develops in the circulating blood plasma, and possibly stimulates the various organs of the body to synthesise or redistribute plasma protein. This view is substantiated by the relative stability of the plasma protein concentration in human females during the ovarian cycle (Danforth, Boyer and Graff, 1946), despite the fluctuations in the concentration of circulating oestrogen.

3. The tissue component.

Modification of the capillaries alone cannot account for the retention in the perineum of 1 to 4 litres of fluid during the first part of the cycle. Each day about 250 ml. of water and several grams of solid material accumulate in the perineum. That is to say, as the water moves through the perineal tissues some mechanism fixes about 250 ml. of water in the tissue every day.

(a) Water and electrolyte metabolism.

In all body tissues there is a continuous turnover of water. Water is constantly entering the tissues from the capillaries. Simultaneously, water is being removed via the venules and lymphatics. These movements constitute the basic water turnover of tissue fluid which may vary in volume and rate. The regulation of this turnover involves vascular, renal, suprarenal, pituitary, skin, bowel and other systems. In addition, the specific nature of a given tissue

is important in regulating the basic water turnover through that tissue.

Superimposed on this turnover is the retention and utilization of water by the tissues. To a certain extent this retention and utilization is independent of the basic water turnover. The two functions may fluctuate in opposite directions. For instance, the baboon sex skin may swell although the animal is dehydrated; alternatively the sex skin may deturgesce although the large volume of water released is not excreted by the kidneys. In certain circumstances, the volume and rate of water turnover may influence the volume and rate of water retained and utilized.

In the study of the baboon perineum both functions should be considered. The swelling of the perineum is primarily an increase in the volume of water retained in the sex skin tissue (Tables 1 and 14). Beyond certain limits, the quantity of water ingested plays no part in determining the volume of fluid diverted into the perineal tissue during turgescence (Gillman and Gilbert, 1956 a, 1956 b, 1956 c, 1956 d). Both the basic water turnover and the retention and utilization of water in the tissues is under metabolic control. In the baboon sex skin there is also an endocrine regulation of these events.

Immediately after the onset of menstruation the rising output of oestrogens causes the sex skin to swell at a constant speed until

the swelling is maximal. The perineum is usually maximally enlarged before ovulation. 24 to 48 hours after ovulation an acute decrease in the size of the sex swelling occurs, associated with the decreasing concentration of oestrogen and the increasing concentration of progesterone. The sex skin rapidly contracts to its resting volume and remains in that state until the next bleeding begins. An increase in body weight and a decrease in body weight occur during the follicular and luteal phases of the cycle respectively. A marked diuresis accompanies the resolution of the sex swelling (Zuckerman, 1930, 1935 a, 1935 b; Allen, 1926, 1927; Hartman, 1927, 1932; Parkes and Zuckerman, 1931; Gillman, 1935, 1937 a, 1937 b, 1938; Gillman and Gilbert, 1946, 1956 a, 1956 b, 1956 c, 1956 d; Gilbert and Gillman, 1944 a, 1944 b; Gillman, Gilbert and Levy, 1952; Clark, 1946, 1947).

Oestrogen also enhances the water content of non-sexual skin. Selye (1944) treated the so-called hairless mouse with topical applications of a solution of oestradiol (25 mg. per cent). The treated animals responded with a non-pitting cutaneous oedema, histologically similar to that which occurs in the sexual skin of monkeys.

Endogenous hormonal stimulation or oestrogen administration leads to an influx of water and electrolytes into the tissues of the female generative tract (Astwood, 1938 a, 1938 b, 1939; Talbot,

Lowry and Astwood, 1940; Carroll, 1945; Cole, 1950; Roberts and Szego, 1947, 1953; Zuckerman, Palmer and Bourne, 1939; Zuckerman, Palmer and Hansen, 1950). Androgens and progesterone antagonise the effect of oestrone in moving electrolytes and water (Roberts and Szego, 1953). Similarly, androgens, progesterone and desoxycorticosterone antagonise the oestrogen effect in the baboon and cause the deturgescence of the sex skin swelling, with the consequent movement of electrolytes and water from the perineum into the circulation (Gillman, Gilbert and Levy, 1952).

A considerable amount of water accumulated in the perineum of the baboon during the follicular phase of the ovarian cycle (Table 14). Moreover, the water accumulates at a constant rate of about 250 ml. per day, irrespective of the water turnover (Gillman and Gilbert, 1956 a). The retention of water therefore, can be ascribed only to a local tissue reaction, the rate of which is the limiting factor in the expansion of the perineum. That is to say, the water is actively maintained in the sex skin, and this active retention is regulated by the ovarian hormones. To explain this retention one may postulate the existence in the perineum of a system having the property of reversibly binding large quantities of water. Such a binding system could be provided by a polyelectrolyte gel, which could bind water under the influence of oestrogen, and subsequently release the water as the hormonal pattern alters.

At this juncture it may be useful to consider some of the general properties of polyelectrolyte gels and their possible significance in the water retention in the sex skin of the baboon during the first part of the ovarian cycle.

According to the nature of the ionizable groups the gels may be of three major types: polyacid, polybase or polyampholyte. Among the biological gels the most studied are the protein gels which belong generically to the polyampholyte type. The other large group of gels is the polyacids, consisting mainly of polysaccharides. To this latter group belong hyaluronic acid and the polysulphonic acids such as chondroitin and mucoitin sulphate (Pigman and Goepp, 1948; Meyer, 1954, 1956 and Meyer, 1950) and heparin. Special note is taken of the inclusion of hyaluronic acid. The suggestion has been made that hyaluronic acid participates in the binding of water in the baboon sex skin. The classical gel forming substance, agar-agar, is a polysaccharide carrying one sulphate group every tenth monomer and also belongs to the group of polyacid gels (Barry, Dillon and McGettrick, 1942; Barry and Dillon, 1944).

Another important group from the biological point of view comprises the gels resulting from the interaction of two or more polyelectrolytes, examples of which are the gel structures of the chromosomes, resulting from the interactions of nucleic acid with protamines and histones (Stedman and Stedman, 1947). The gels of the connective tissues, produced by the interaction of procollagen with hyaluronic, chondroitin

sulphuric and mucoitin sulphuric acids (Meyer, 1947) and the constituents of muscle derived from the interaction of actin and myosin (Szent-Gyorgyi, 1947; Mommaerts, 1950) belong to this class of complex gels.

A necessary condition for the formation of a gel is the existence of a cross-linked network. The cross-linking may take place by covalent bonds, by van der Waal's forces or by electrovalent links. The network may grow from polyfunctional monomers during polymerisation, or may result from the junction of polymeric chains, as is usual with biological gels.

The main covalent bonds of the protein chains in biological gels are the S - S bonds (Speakmann, 1931), amide bridges between the amino and carboxylic groups situated on neighbouring chains (Bjorksten, 1951) and ester and ether links (Katchalsky, 1954). The stability of the primary cross links makes the network robust and almost independent of the nature of the imbibing medium and of inner stresses. The network is not torn apart by absorbed solvent and the swelling of these gels does not lead to ultimate dissolution of the network. Swelling, even in the best solvents, is always limited and reaches a state of "equilibrium swelling". The sex skin swelling of the Chacma baboon also has a limiting size. Excess oestrogen or water and electrolytes does not increase the size of the swelling beyond this limit. Many covalent bonds in a polyelectrolyte gel may well be the explanation of the limited maximum perineal size.

The swelling of the sex skin may also be limited by the tension of the epidermis and fibrous strands and by metabolic control of the blood vessels and connective tissue cells.

The formation of biological gels by van der Waal's forces and hydrogen bonds is by now well established. Many physical properties of such gels demonstrate the lability and reversibility of the van der Waal's linkages. The swelling of the baboon sex skin is very labile and easily reversible. Decrease in oestrogen or relative excess of progesterone causes a deturgescence of the perineum.. The end result of the hormonal action may be a breaking of van der Waal's forces and hydrogen bonds.

The classical example of electrovalent bonding in polyelectrolyte gels is provided by the proteins at their iso-electric points. Attractive forces operate in gel formation of proteins when the molecules carry approximately equal numbers of positive and negative charges.

The contribution of the charged groups to the stabilization of the network is determined not only by the number and valency of these groups but also to a large extent by their spatial arrangement in the chain units and in the network. Equality in number of positive and negative charges is not sufficient to produce efficient bonding. Thus in randomly arranged fibrillar molecules of the gelatin type the overall electrostatic attractions at the iso-electric point will

be negligible as compared with other forces. On the other hand, in highly organised collagen, in which the natural arrangement of the network elements provides for close proximity of positive and negative groups, the electrostatic attraction plays a predominant role in the organisation of the fibres. In the perinea of the six baboons examined, collagen was present in quantities of from 1 to 10 g. During swelling the microscopic appearance of the collagen fibres alter (Gillman, 1956). The collagen fibres may provide a specifically organised skeleton onto which a mucopolysaccharide may fit itself. Consequently, the mucopolysaccharide will be a highly organised polyelectrolyte capable of reversibly binding large quantities of fluid during the ovarian cycle.

In attempting to analyse the electrostatic contribution to the stability of the more complicated biological gels, it is necessary to consider the influence of three variables; the degree of ionization of the different groups, which may be regulated by the exterior pH; the dielectric constant of the medium, which constant may be varied by the polarity of the solutes in it; and the salt concentration of the external solution.

The swelling of gelatin gels increases when the pH is shifted from the iso-electric point either to the alkaline or to the acid side. An analogous swelling occurs with collagen (Bowes and Kenten, 1950).

This increase in swelling capacity may be attributed to two factors. The first depends on the accumulation in the gel of "counter" ions of the alkali or acid which penetrate the network and neutralize the fixed charges on the protein molecules. This accumulation of small ions in the gel increases its osmotic pressure and makes it swell by absorption of water. The other factor depends on the change of the electrostatic potential of the macromolecules. Shifting the pH from the iso-electric point diminishes the number of attracting intermolecular zwitterion pairs and causes the attraction potential to be slowly replaced by one of repulsion. The number of electrovalent bonds therefore decreases and the network is loosened, permitting higher degrees of swelling.

The cells of the glomerular tubules regulate the pH of the urine. The cells of the perineal connective tissue may possess the ability to regulate the hydrogen ion concentration of the fluid in the vicinity of a polyelectrolyte gel, thus modifying the degree of the gel's swelling.

Depressing the dielectric constant of the aqueous medium increases the electrostatic potential and strengthens the long range orientating forces. The polarity of protein surfaces is capable of altering the dielectric constant of the adjacent fluid medium. Suitable apposition of protein molecules may therefore modulate the structure of a polyelectrolyte gel.

The influence of neutral salts on polyelectrolyte gels is of various kinds. The concentration of neutral salts may play a decisive role in the transformation of sols into gels. The Donnan distribution of salt between the gel and the solution will be a prominent factor in determining the physical properties of the gel. These effects, apart from their purely electrostatic character, determined simply by the charge of the ions, are also specific, as expressed by the Hoffmeister series. The most important general effect of neutral salt is the formation of Debye atmospheres around the charged groups of the polymeric chains with a subsequent diminution of the electrostatic potential. In the case of attraction at the iso-electric point, the addition of salt decreases this attraction. The number of network junctions diminishes. Correspondingly, the swelling at the iso-electric point increases. On the other hand, in the pH region of electrostatic repulsion, the screening effect of the salt weakens the repulsion. Therefore a larger number of crosslinks, say of the van der Waal's type, is made possible. Consequently a decrease in swelling takes place.

Highly ionized polyelectrolyte molecules exert sufficiently strong attraction on polyvalent ions of opposite charge to cause association between the small ions and the polymeric chain (Bjerrum, 1926). Thus polyvalent ions influence the number of cross links not only by changing the potential of the polyelectrolyte but also by participating directly in the network junctions.

Gelation of polyuronic acids by polyvalent ions is of great practical importance. In particular, the gelation of hyaluronic acid may be influenced by polyvalent ions. The calcium ion associated with polyuronic acids may be exchanged with an excess of monovalent salt and from the exchange equilibria the strength of the bond can be estimated.

The swelling of protein gels is determined mainly by the osmotic pressure difference between the ions in the gel and those outside (Proctor, 1914; Proctor and Wilson, 1916). Yet the estimate of the osmotic contribution of the counter ions and other small ions does not follow from the classical laws of ideal solutions. Even in the case of very dilute polyelectrolyte gels the deviations from the ideal are very pronounced. The osmotic co-efficient of sodium ions in the presence of ionised polyacrylic acid may drop to as low a value as 16 per cent of the ideal (Kern, 1938 a, 1938 b, 1939). Decreased osmotic activity of the counter ions is due to the attraction of the powerful electrostatic field of the macromolecule.

Oestrogen is capable of altering the ionic composition of tissue fluid (Roberts and Szego, 1953). The hormones may similarly regulate the ionic composition of the perineum. Consequently, oestrogen may modulate the properties of a polyelectrolyte gel in the perineum by altering the ionic composition of the tissue fluid.

It is suggested that in the sex skin there is a polyelectrolyte gel to which large quantities of water could be bound. In the early part of the ovarian cycle the gel may imbibe water and electrolytes. In addition, the imbibed electrolytes could draw more water into the gel by virtue of the osmotic gradient. The proteins would have adequate opportunity to diffuse evenly through the gel because the imbibition would take place over a period of days. The overall energy with which the water would be bound and the internal cohesion of the gel matrix will limit the degree of swelling. A constant interchange of water, electrolytes and proteins would occur between the bound and unbound segments. The unbound segment would constitute part of the normal water turnover, which in the circumstances may well be augmented. After ovulation the bound water and electrolytes would be more or less rapidly released and would then be part of the continuing water and electrolyte turnover.

The binding system may function through one of two main types of mechanisms. The retention and subsequent release of water may be effected by a change in the properties of the system or by an increase in its mass. Changes in the properties of the binding system could be of four main types. Firstly, there may be a change in the molecular structure or composition of the binding material. Secondly, as has been shown, the degree of ionization of the groups in the molecule may change, possibly due to some change in the pH of the external solution. Thirdly, the polarity of the solutes in

the imbibing medium may alter the dielectric constant of the latter. Fourthly, the salt concentration of the external medium may vary. Anderson (1956) has suggested that oestrogen may alter the electrolyte composition of perineal tissue fluid. Oestrogen could thus modify the polarity of the binding system, making it more hydrophilic in the first part and more hydrophobic in the second part of the cycle. Whatever the mechanism, it is integrated into the metabolism of the tissue, and it is regulated by the gonadal hormones.

An increase in the mass of the binding system may be due to oestrogenic stimulation of synthesis or to oestrogenic inhibition of destruction. The latter may operate via oestrogenic inhibition of the enzyme system responsible for destroying the binding material.

Proteins, nucleoproteins, polysaccharides and mucoproteins possess the properties requisite for the postulated binding system.

Unequivocal evidence of the presence of nucleic acids in the perineal fluid was not found. However, the administration of oestrogen to many mammals initiates an increased protein and nucleic acid synthesis in the reproductive tract (Roberts and Szego, 1953).

Appreciable quantities of a mucinous polysaccharide accumulate in the sex skin of the female monkey during the oestrus phase of the menstrual cycle. The mucin is viscous, stringy and is precipitated by acetic acid (Ogston, Philpot and Zuckerman, 1939). Testis hyaluronidase causes an immediate drop in the viscosity of the mucin

solution, a loss of precipitability by acetic acid and the liberation of reducing substances consisting of N-acetyl-glucosamine and glucuronic acid. The mucin of the sexual skin of monkeys was therefore thought to be hyaluronic acid or a closely allied substance (Chain and Duthie, 1940). The inoculation of hyaluronidase into the sex skin of the monkey at first brings about a softening of the tissue which pits under pressure and later collapses entirely. The complete disappearance of metachromasia is the most conspicuous histochemical finding in the sex skin injected in vivo or treated in vitro with hyaluronidase (Duran-Reynals, Bunting and Van Wagenen, 1950). The suggestion has been made that the metachromasia is due to a mucopolysaccharide containing both hyaluronic acid (Boas, 1949; Boas and Ludwig, 1950; Ludwig and Boas, 1950) and chondroitin sulphuric acid (Schiller, Benditt and Dorfman, 1952). In combination with proteins, hyaluronic acid constitutes the mucinous component of the connective tissue ground substance generally (Asboe-Hansen, 1954). The acid polysaccharides have been implicated in the binding and transfer of small ions and of water (Kulonen, 1952; Paulson, Sylvan, Hirsch and Snellman, 1951; Ropes and Bauer, 1953; Sylvan, Paulson, Hirsch and Snellman, 1951). Although hyaluronidase can cause the collapse of the monkey sex skin it may not be the factor operative in vivo. Substances other than hyaluronidase give spreading reactions; for example, factors in mammary glands, thyroid, azoproteins,

mercurial diuretics and ascorbic acid (Duran-Reynals, 1954). In general, the evidence suggests that hyaluronic acid may be an integral part of a polyelectrolyte gel which would permit reversible expansion and contraction of the sex skin in the monkey. By analogy, oestrogen may increase the concentration of hyaluronic acid in the sex skin of the baboon. Hyaluronic acid in other parts of the body appears to function in water binding, as a lubricant and as a shock absorber (Meyer, 1954). The structure of hyaluronic acid, as a highly hydrated coiled sphere easily deformed by flow and easily 'drained', appears to explain well these biological functions. However, the baboon sex skin may differ from that of the monkey. The baboon may elaborate a polysaccharide different from hyaluronic acid. Already six different polysaccharides have been isolated. They are (Meyer, 1954):

1. hyaluronic acid,
2. chondroitin sulphate A,
3. chondroitin sulphate B,
4. chondroitin sulphate C,
5. chondroitin, and
6. keratosulphate.

Chondroitin sulphuric acids possess no particular viscosity and unlike hyaluronic acid, only a slight capacity to bind water (Asboe-Hansen, 1954). A variety of acid polysaccharides exist and

it is possible that the polysaccharide in the perineum of the baboon is of a new type.

Alternatively, the mechanism of sex skin swelling in the baboon may not involve a polysaccharide at all. The gross behaviour of the sex skin of the baboon is markedly different from that of the monkey. Whatever the nature of the polyelectrolyte gel in the baboon sex swelling, it is regulated by the ovarian hormones, oestrogen and progesterone.

The oestrogen or an oestrogen metabolite may act directly on the binding substance the properties of which are thereby regulated. The oestrogen may inhibit the destruction of the binding system, or may stimulate its production or finally oestrogen may alter the physico-chemical properties of the binding system. The withdrawal of oestrogen leads to the subsidence of the swelling. Thus, oestrogen must be constantly present to maintain a specific balance of several different relevant reactants. If the binding material is hyaluronic acid, oestrogen may inhibit the synthesis or activity of hyaluronidase. Oestrogen is known to suppress the spreading reaction of hyaluronidase (Sprunt, 1950). By contrast, progesterone and androgens may promote synthesis or activate the enzyme.

Alternatively, oestrogen may regulate cellular activity. Cellular activity, in turn, may alter the properties or rate of

synthesis of the binding substance. Oestrogen is known to influence cellular activity (Roberts and Szego, 1953). Oestrogens are deposited in the active sex skin (Fisher, Krohn and Zuckerman, 1936), but their influence on the activity of perineal cells is unknown.

From the available evidence, however, it can be asserted that the gonadal hormones do regulate the metabolism of the sex skin. A polyelectrolyte gel may form an integral part of the sex skin physiology, regulated by the gonadal hormones and responsible in part for the swelling of the sex skin.

4. The vascular component.

(a) The vascularity of the perineum.

The vascular system is the main portal of entry of substances into the perineum. The tissue is not unusually vascular when at rest. When swollen the tissue is noticeably avascular, although the blood supply to the dermis is increased as is evident from the pink colour and warm surface. The blood vessels are coiled during the resting state and as the perineum expands the vessels uncoil and thereby supply the entire swollen tissue (Gillman, 1956). The uncoiling of the blood vessels explains the avascularity of the subdermal tissues evident at maximal turgidity. Some vascular proliferation during perineal expansion has been observed (Gillman, 1956). A small increase in insoluble nitrogen mass is therefore expected when the perineum passes from the resting to the expanded state.

The deeper vessels of the subcutaneous layer of the sex skin of the monkey, *Macacus rhesus*, are larger and contain more blood than the vessels in the skin elsewhere over the body. A direct correlation exists between the degree of reddening, the size of the superficial vessels and their degree of engorgement (Collings, 1926).

In his discussion, Collings (1926) observed;

"This stimulus (to engorgement) may be directly upon the vessels, but it seems more likely that the control is effected through a vasomotor mechanism, as is the case in erectile tissue."

The perineum is innervated by the same nerves as the external genitalia, including the penis (Langley, 1890; Langley and Sherrington, 1891; Sherrington, 1892). Nervous regulation of muscular veins in the penis participates in penile erection. The main veins leaving the perineum are unusually muscular (Gillman, 1956). Muscular contraction would cause a greater hydrostatic pressure which could lead to a more rapid formation of tissue fluid.

Zuckerman (1934) produced evidence that neural lesions disturb the rhythm of the menstrual cycle in monkeys. On the other hand, oestrin acts as readily and as well on the denervated as on the normal sex skin (Zuckerman, 1935 b). Zuckerman (1935 b) postulated a physiological balance in the normal animal between sympathetic and

parasympathetic control of at least the central sex skin vessels. Similarly, in the completely denervated preparation there is a parallel loss of both these controls. In both cases, the sex skin response was a balanced one from the point of view of nervous influences. On the other hand, if one side of the autonomic mechanism were to sufficiently overpower the other, the manifestations of sex skin activity might be different from those which would appear were the other side dominant. Zuckerman (1935 b) reported such observations, but they have not yet been confirmed.

Oestrogen is known to affect the vascular system in many other tissues. Stimulation of the uterus and vagina by oestrogen causes hyperaemia of those tissues (Markee, 1932; Holden, 1939; Freed and Lindner, 1941; Hechter, Krohn and Harris, 1941, 1942; Reynolds, 1949; Richter and Albrich, 1952). The oestrogenically produced hyperaemia in the monkey sex skin is partly, at least, a local phenomenon (Chamberlin, Gardner and Allen, 1941), although inunction of oestrogens on the sex skin of the Chacma baboon produces no hyperaemia (Gillman, 1956). The oestrogens may, however, act directly on the capillaries of the sex skin of the Chacma baboon.

Three different and possibly dissociated effects may result from the action of oestrogen on the perineal capillaries. Firstly, there may be uncoiling of capillaries. Secondly, the uncoiling may be associated with or independent of proliferation of new capillaries.

Thirdly, there may be alterations in the capillary permeability. Although the three phenomena are integrated they may be modulated by different regulations. Under specific physiological conditions the three events may vary independently of one another, thus altering the degree of participation of the several events in the swelling of the perineum.

(b) The permeability of the perineal capillaries.

In addition to the uncoiling and proliferation of the capillaries, a high degree of capillary permeability is required to allow the movement of 1 to 4 litres of fluid, about 40 g. of protein and quantities of carbohydrates, lipids and electrolytes.

Some evidence is available to support the contention that the permeability of the perineal vessels is the same as in other connective tissues of the body. The protein concentration of perineal tissue fluid (Fraction A) was from 1.7 to 3.2 times lower than the corresponding concentration in the blood plasma (Table 11). The concentration of the proteins of rabbit muscle interstitial fluid is about 3.2 times lower than in the corresponding rabbit blood plasma (Gitlin and Janeway, 1954). The capillaries in the perineum of the baboon apparently possess a permeability similar to the capillaries in rabbit muscle.

The expanding perineum retains about 250 ml. of water per day (Gillman and Gilbert, 1956 a). The volume of water moved through the quiescent sex skin daily is probably several litres. That is

to say, the permeability of the connective tissue capillaries, in general, is more than sufficient to allow the passage and the retention of 250 ml. of water per day. The permeability of the capillaries of the connective tissue of the perineum therefore, may be substantially the same as the permeability of capillaries in other connective tissues.

The average rate of disappearance of Evans Blue dye (T-1824) in the first hour after injection into the peripheral blood of the Chacma baboon is high in the first half of the follicular phase and low for the remainder of the follicular phase, only to rise again during the luteal phase (Cohen, 1953). Hence plasma albumin permeates more rapidly through the capillaries during the first half of the follicular phase and during the luteal phase. These variations in permeation of albumin may result from alterations in the size of the functional capillary bed or from variations in capillary permeability which may be general or confined to the reproductive tract and perineal tissues.

Administration of oestrogen to castrated rats and rabbits increases the permeability of uterine and vaginal vessels (Hechter, Krohn and Harris, 1941, 1942).

Although more direct evidence is necessary to establish the precise regulation of perineal vessels by oestrogen, the evidence available suggests that oestrogen not only regulates the vascularity

of the perineum, but that it also modulates the permeability of the capillaries of the sex skin.

5. Conclusion.

The reactions in the baboon sex skin probably occur in the following way.

The ovarian hormones, oestrogen and progesterone, are the primary regulators of the phenomena described.

Oestrogen dominates the hormonal pattern in the first part of the cycle. The oestrogen determines the observed reactions in the cells, fibres and the tissue fluid. The tissue response modifies the properties of the tissue gel in such a way that water is absorbed by the gel. At the same time, the tissue becomes more vascular, capillaries uncoil and there may even be an increase in capillary permeability. As a consequence, the flow of fluid through the tissue may be greatly increased.

The rising concentration of oestrogen permits the gel to retain about 250 ml. of fluid per day. This retention of fluid is not a function of the total water metabolism of the organism. During the follicular phase the animal goes into positive water balance. However, the volume of water passing through the perineum greatly exceeds the volume retained. Therefore, the volume of water retained in the sex skin is not limited by the water turnover of the animal as a whole. However, the rate of water retention in the sex skin

is limited to a maximum of 250 ml. per day. Therefore, a limiting reaction presumably operates as part of the water-retaining mechanism. This limiting reaction may be associated with the expansion of the gel system. The gel system can expand sufficiently to absorb up to about 1 to $1\frac{1}{2}$ litres of fluid and, provided oestrogen is available, the gel is sufficiently stable to retain the water indefinitely. In addition, a framework of organised collagen or cells and their processes would impose on the gel a high degree of organisation, which may contribute to the expansion of the gel system.

The perineum swells at a constant rate until it achieves its maximum size near the end of the follicular phase of the cycle. For a variable period thereafter the swelling is maintained at its maximum size by the persistent presence of oestrogen. During this period the perineum is in an unstable dynamic equilibrium, neither increasing nor decreasing in size. This dynamic equilibrium is immediately dependent on the presence of oestrogen. A decrease in the concentration of oestrogen is accompanied by a decrease in the volume of the tissue gel.

The composition of the tissue fluid in the turgid perineum is not the same as that of the blood plasma, possibly the consequence of the specific metabolic activities of the capillary endothelium and of the tissue cells. The concentration of plasma proteins in the tissue fluid remains at approximately half that of blood plasma. Although the capillary endothelium does allow all the various fractions of the

plasma proteins to pass through into the tissues, the plasma proteins are not treated equally by the endothelium.. The proportion of globulins in the tissue fluid is lower than in the blood plasma, possibly due to a slower transfer of globulins across the capillary membrane.

The tissue fluid is formed slowly and continuously. The proteins of the turgid sex skin are equally distributed throughout the tissue fluid both in the water bound to the gel and in the unbound water. The capillaries apparently participate in maintaining the concentration of protein in the tissue fluid at a fairly stable level by regulating the influx of plasma proteins. The protein in the tissue fluid is derived directly from the blood plasma. The removal of plasma proteins from the circulation is associated with a relative hypo-proteinaemia, which in turn may be correlated with a possible increase in plasma protein synthesis, a decrease in protein catabolism and a redistribution of plasma protein.

The supervention of ovulation fundamentally alters the hormonal pattern. The concentration of progesterone rises. A new regulation is established which is dominated by progesterone. In an anovulatory cycle where progesterone is not usually elaborated, the new hormonal pattern is dependent on a decreasing concentration of oestrogen. Whether progesterone is present together with oestrogen or whether the progesterone concentration is low accompanied by a fall in the oestrogen concentration, the gross effect on the enlarged sex skin is a decrease in the size of the sex skin.

The changing hormonal pattern in either case induces a profound change in the tissue metabolism and initiates a new set of events. The properties of the tissue gel are radically altered. Instead of absorbing water the gel system rapidly releases the bound water. The unbound water forms part of the free tissue water and passes out of the tissue through unusually large lymphatic channels which have opened up. The large volume of free water, electrolytes, proteins and other substances passes rapidly into the lymphatics and venules. Within several days the gel is resolved. The dermis recedes and the capillaries coil while some of them even atrophy and disappear. As the gel resolves, the sex skin swelling returns to its resting volume and remains in that state until the next bleeding begins.

The diminution in the size of the gel is accompanied by the egress of fluid from the perineum. The solutes, proteins included, pass into the circulation together with the water. The outflow of protein from the perineum can be correlated with a sharp hyperproteinaemia mainly due to the globulins, possibly because they leave the circulation more slowly than do the albumins. The albumins are rapidly distributed from the perineal tissue fluid to the blood plasma and further to the interstitial tissue fluid of the whole body. The hyperproteinaemia may be associated with a depression of protein synthesis and a new distribution of the plasma proteins, with proteins being deposited in the cells and in the tissue spaces throughout the body. The water release is dissociated from the general water metabolism of the

organism. During this period the animal exhibits a negative water balance. Nonetheless, the water turnover of the animal is markedly increased.

The large volume of water and electrolytes released from the perineum increases the circulating volume and stimulates a diuresis. Most of the fluid from the sex skin is eventually excreted.

As with the water metabolism, so, too, the protein metabolism of the perineal tissue is dissociated from the general protein metabolism of the organism. During the early luteal phase of the cycle when the plasma proteins from the sex skin are dislodged into the circulation and a hyperproteinaemia is evident, the dietary protein intake of the animal rises sharply.

The animal passes once again into a premenstrual state. Involution of the corpus luteum in an ovulatory cycle and the development of a new ovarian follicle once again initiate an 'oestrogen' hormonal pattern with the characteristic increase in the volume of the tissue gel and with the associated increase in plasma proteins, water and electrolytes.

The retention of these metabolites is thus directly under the influence of oestrogen. On the other hand, after ovulation the resolution of the gel and the removal of the protein, water and electrolytes from the tissue is directly controlled by progesterone. Thus we conclude that the cyclic behaviour of the tissue gel and of the plasma proteins, water and electrolytes contained in the gel, are directly regulated by the ovarian hormones.

SECTION E

SUMMARY

The study of the relationship between the blood plasma proteins and the tissue proteins was conducted upon 14 animals belonging to the species *Papio ursinus*, using the perineal tissue of this animal as an example of connective tissue. The conditions under which the baboons are maintained within the experimental colony were described. The physiological regulation of the rhythmical swelling and deturgescence of the perineum was recorded.

Methods for obtaining samples of perineal tissue, and for fractionating these samples were detailed. The Biuret and micro-Kjeldahl methods were employed for quantitative protein and nitrogen determination respectively. Plasma protein fractionation was achieved by means of a sodium sulphite method as well as by electrophoresis. The tissue was separated into components, soluble and insoluble in 0.9 per cent sodium chloride respectively. Collagen concentrations were determined by virtue of its specific hydroxyproline content.

The protein and water content of the perinea of six adult female *Chacma* baboons was determined at the stages of minimal and maximal sex skin swelling. In all the estimations performed, wide interindividual variations were observed.

The mass of the perineum fluctuated widely during the ovarian cycle. At perineal rest the mass of the perinea in the six animals was shown to vary between 143 g. and 275 g. By contrast, at maximum turgescence

the mass of the perinea rose to between 1230 g. and 4040 g. The increase in mass varied from 1070 g. to 3770 g. in different animals.

Against the background of this phenomenal increase in tissue mass, the behaviour of the nitrogen and protein in the tissue was examined.

The mass of nitrogen in the perinea increased by about 7 g. and 23 g. in different animals. Most of this increase was due to soluble nitrogen which contributed an increment of about 6 g. and 15 g. in different animals. A very small, and possibly insignificant, increase was observed in the mass of insoluble nitrogen. The concentration of soluble nitrogen did not vary significantly during the menstrual cycle.

The mass of hydroxyproline in the tissue was constant during the menstrual cycle, suggesting that the protein of the collagen fibres did not increase in mass as the tissue swelled.

By gentle centrifugation, the perineal tissue was separated into two fractions. These were:-

1. Fraction A, a liquid, presumably derived mainly from the tissue fluid; and,
2. Fraction B, the solid residue which consisted of collagen and elastic fibres, cells, nerves and blood vessels.

Fraction A, the tissue fluid, contained between 4 g. and 6 g. of protein in different animals during perineal rest. When the perinea were swollen the tissue fluids contained between 27 g. and

110 g. of protein. Four different animals evinced increases of about 40 g. in protein mass and one animal showed an increase of about 105 g. The increase in protein mass in the tissue fluid mainly accounted for the increase in total nitrogen in the whole tissue. At the same time there was no consistent variation in the protein concentration of the tissue fluid.

The proteins in the tissue fluid and those in the blood plasma yielded similar paper electrophoretic patterns. Both protein solutions could be fractionated by 28 per cent sodium sulphite. From this, and from the evidence of another investigator, it was concluded that the protein in the perineal tissue fluid is plasma protein.

The concentration of total protein in the blood plasma was from 1.7 to 3.2 times higher than the concentration in the corresponding tissue fluid. The proportion of albumin in the tissue fluid was greater than that in the blood plasma.

The major portion of the increased mass of the perinea was caused by an increment in water content. The water in the perinea contributed about 1000 g. and 3620 g. to the swelling in different animals. In one instance the water content increased by about 7 times and in another by 16 times. Solid constituents of the perinea added from 60 g. to 150 g. to the total increase in mass in the different perinea.

In all six animals the body weight varied rhythmically with the mass of the perinea. Most of the increase in body weight was located in the perineum. The bulk of the increase in perineal mass was due to water derived from the diet.

The experimental work has shown that quantities of proteins accumulate in the perineum during the follicular phase. The source of this new perineal protein was discussed. The available evidence militates against a de novo synthesis in the perineum of this new protein. The protein which accumulates in the perineum was considered to be plasma protein and to have its origin from circulating plasma proteins. It was suggested that during the follicular phase, plasma protein synthesis is increased, and catabolism is decreased, with a redistribution of plasma proteins from the intra-cellular, extra-vascular and intra-vascular compartments to the intercellular spaces of the perineum. It was suggested that these metabolic changes are associated with the withdrawal of plasma proteins from the circulation and their retention in the perineum.

The accumulation of fluid in the perineum is presumably due to a fluid-retaining mechanism. A polyelectrolyte gel, possibly containing an acidic polysaccharide, may operate in the retention of water in the enlarging sex skin. The properties of polyelectrolyte gels and their relevance to the baboon perineum were discussed.

Vascular changes are evident during perineal swelling and deturgescence. The vascularity of the perineum and the permeability of the perineal capillaries possibly increased during turgescence.

A brief description of the events in the perineum during the ovarian cycle ends the discussion. The perineal sex-swelling is regulated directly by the ovarian hormones, oestrogen and progesterone, which regulations constitute the opposing phenomena of the central dynamic process upon which is dependent all the other events in the perineal cycle.

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