CHEMICAL CHANGES IN THE UTERUS OF THE RAT DURING LATE PREGNANCY AND POST-PARTUM INVOLUTION. THE EFFECTS OF LACTATION AND HORMONE TREATMENT

R. A. GRANT

A.R.C., Institute of Animal Physiology, Babraham, Cambridge

(Received 11th August 1964)

Summary. A study has been made of changes in the amount and concentration of some chemical constituents of the rat uterus during the latter part of pregnancy and post-partum involution in both lactating and non-lactating animals. Dry, defatted, uterine tissue was analysed for hexose, hexosamine, uronic acid, nucleic acid and collagen. Samples of serum were analysed for free hydroxyproline and serum mucoprotein.

Up to the 3rd day *post partum* the rate of uterine involution (by weight) was similar in both lactating and non-lactating rats. During this period the overall rate of tissue loss was found to be a first order process kinetically, the fraction of tissue lost per day being about one-third. Thereafter there was a marked divergence in the two groups; hyperinvolution occurred in the lactating group but not in the non-lactating animals.

The disappearance of collagen showed a distinct lag during the first 24 hr *post partum* in both groups. At 5 to 8 days *post partum*, uteri from non-lactating animals contained 12% more collagen than non-pregnant controls and about 100% more than uteri from lactating rats.

The total hexose content showed the largest relative decrease *post partum* of all the chemical components studied; the hexose concentration fell sharply during involution to about half the value found on the 20th day of pregnancy. Hexosamine and uronic acid concentrations, however, showed only slight decreases while the collagen concentration varied considerably during the involution period. The serum hydroxyproline showed a slight increase *post partum* in contrast to the serum mucoprotein level which was markedly elevated during the latter part of pregnancy and the whole of the involution period studied.

Administration of oestrone prevented hyperinvolution in lactating rats.

INTRODUCTION

During recent years a number of chemical and histological studies have been made on the rat's uterus during pregnancy and in the period of post-partum involution (Harkness & Harkness, 1954; Harkness & Moralee, 1956; Woessner,

1962; Fainstat, 1962, 1963). However, these investigations have been mainly concerned with the formation and breakdown of collagen; the other chemical components of the uterus have been largely neglected. It therefore seemed of interest to investigate changes in some of the other uterine constituents and to compare the results with the findings for collagen. This study has been concerned with variations in the wet and dry weights of the uterine horns and changes in their content of hexose, hexosamine, uronic acid and nucleic acid during the latter part of pregnancy and up to the 8th day of post-partum involution. The results have been expressed in terms of concentration (% of dry fat-free tissue) and total amount; for comparison a similar series of analyses was made for collagen.

The hexose content, determined by anthrone after hydrolysis of the tissue with dilute mineral acid, represents the tissue glucose plus the hexose stored as glycogen and gives an estimate of the available energy reserve of the organ. Hexosamines and uronic acids occur as constituents of mucopolysaccharides and, as such, form part of the ground substance of the tissue. The serum mucoprotein level is known to be elevated during pregnancy and after labour in humans (Shetlar, Kelly, Foster, Shetlar & Everett, 1950; Gillman & Pillay, 1959). In view of this, serum mucoproteins were analysed in order to detect any possible relationship with the mucopolysaccharide content of the uterus.

It has been found, by histological examination, that although the size of the uterine muscle cells varies widely during pregnancy and post-partum involution, the number of cells does not appear to change (Maibenco, 1960; Kuramitsu & Loeb, 1921; Dawson, 1946). In view of the apparent change in the volume ratio of nuclei to cytoplasm, analyses were made for the nucleic acid content of the uterus.

Woessner (1962) noted in the rat a slight increase in the serum hydroxyproline in the period 0 to 96 hr *post partum* compared with the value found after this time. In the present study an attempt has been made to correlate the serum hydroxyproline level with the loss of collagen from the involuting uterus.

Hyperinvolution (decrease in the size of the uterus below the non-pregnant level) does not occur in the rat uterus (Kuramitsu & Loeb, 1921), or in the cat (Dawson, 1946), if lactation is prevented. Its occurrence has been correlated with lack of oestrogens arising from inactivity of the ovarian follicles during lactation. It would appear that this mechanism is neurohormonal and effected *via* the pituitary and ovaries. In view of this known effect of lactation on uterine involution a second series of analyses was carried out on uteri from rats in which lactation had been prevented by removal of the litter at birth. The term 'non-lactating' will be used in this paper to refer to a rat from which a litter has been removed immediately *post partum*.

The effect of several hormones on the degree of post-partum involution was also studied.

METHODS AND MATERIALS

Female rats of the Wistar strain were killed on the 15th and 20th days of pregnancy and on the 1st, 2nd, 3rd, 5th and 8th days *post partum*. One group of

animals was allowed to suckle their litters during the post-partum period. The number of rats in each group was five except for the 8th day post-partum group which numbered eight. All animals were *prima gravidae*. A second series of animals was also killed on the above days *post partum*; in this series each group consisted of three animals and the litters were removed at birth. A further group of five non-pregnant control animals was also studied.

The rats were anaesthetized with ether and blood collected by cardiac puncture; the blood was allowed to clot and the serum was separated for serum hydroxyproline and mucoprotein determinations. The uterus and cervix were dissected out and, in the case of gravid uteri, the foetuses and placentae were removed. The cervical portion of the specimens was discarded and the uterine horns lightly blotted and immediately weighed. A small portion of each horn was taken for histological study and the residue re-weighed. A further portion (about one-third of total tissue) was removed for the determination of saline soluble collagen. The residue was re-weighed and placed under acetone (two changes) and then under ether-alcohol, 1:1 (v/v), and finally dried to constant weight in a hot air oven.

In a third series of experiments, lactating and non-lactating animals were given daily subcutaneous injections of oestrone, progesterone, prolactin or oxytocin for the first 5 days *post partum*. The animals were killed on the 8th day and the uteri removed and weighed.

Collagen was determined in approximately 25 mg portions of dried tissue by the method of Neuman & Logan (1950a, b).

Hydrolysis. Portions of dry tissue (50 mg) were heated with 4 ml of 0.5 N-HCl for 4 hr at 100° C in sealed tubes. The solutions were then filtered and the volumes adjusted to 10 ml.

Hexoses were determined in 1 ml portions of the hydrolysate with anthrone according to the method of Trevelyan & Harrison (1952), the results being expressed as glucose. Tests with glucose showed that the amount of destruction during the hydrolysis period was insignificant.

Hexosamines were determined by a modification (Grant, Hathorn & Gillman, 1960) of the Elson & Morgan method (1933).

Uronic acid was determined in 1 ml portions of the hydrolysate by the method of Dische (1947). Glucuronolactone (L. Light & Co. Ltd) was used as a standard.

Serum mucoprotein was determined according to the turbidimetric micro-method of de la Huerga, Dubin, Kushner, Honig, Dyniewicz, Bronsky & Popper (1956). Crystalline bovine serum albumin (Armour) was used as a standard.

Serum hydroxyproline (free) was estimated after precipitation and removal of the serum proteins by addition of an equal volume of 10% trichloroacetic acid followed by centrifugation. The hydroxyproline in the supernatant was determined by the Stegemann assay (1958) modified according to Woessner (1961).

Nucleic acid. An estimate of the total nucleic acid content was obtained by dissolving a portion (about 20 mg) of the dried tissue in 1 ml of 5 N-NaOH. To this solution was added 9 ml of 5% perchloric acid and the mixture was heated at 90° C for 15 min. After cooling and centrifuging down the precipitated

protein, the absorption at 260 m μ was determined in a Hilger Uvispek spectrophotometer. A commercial preparation of nucleic acid (Roche Products Ltd) was used as a standard.

Hormone treatment. Groups of four rats (lactating and non-lactating) were given daily subcutaneous injections of oxytocin (Pitocin, Parke, Davis & Co) 1 unit, prolactin (Armour) 0.66 mg, progesterone 2 mg, or oestrone 100 μ g. The oxytocin and prolactin were given in aqueous (0.9% saline) solution. The oestrone and progesterone were dissolved in arachis oil.

TEXT-FIG. 1. Mean total weights of (a) wet and (b) dry rat uterine horns and (c) percentage solids content (fat-free). $\circ$, Non-lactating; $\bullet$, lactating. NP = non-pregnant.

RESULTS

Changes in total mass of uterine horns

The average total wet weight of the uterine horns on the 20th day of pregnancy was 2.33 g representing an increase of 540% above the non-pregnant value, which agrees closely with the 560% increase reported by Harkness & Harkness (1954). In both lactating and non-lactating animals, the weight of

the uterine horns, expressed either on a wet or dry basis, decreased rapidly *post partum*, this process appearing to commence immediately after parturition (Text-figs. 1a and 1b).

From parturition until the 3rd day *post partum*, the logarithms of the wet and dry weights of the uterine horns fell on parallel straight lines (Text-fig. 6a) the mean slope being 0.172 ($\log_{10}$), and the daily decrease in weight, expressed as a fraction, being almost exactly $1/3$. During this time there was no discernible difference in the rates of involution of the two groups. After the 3rd day *post partum* the rate of involution decreased in both groups; there was, however, a

TEXT-FIG. 2. Mean total content of (a) collagen, (b) hexose, (c) hexosamine and (d) uronic acid in uterine horns. ○, Non-lactating; ●, lactating. NP = non-pregnant.

marked difference between the lactating and the non-lactating animals after this time. Uteri from non-lactating rats were significantly heavier than those from the lactating group at 5 and 8 days *post partum* ($P < 0.05$ and $P < 0.01$ respectively).

In the case of lactating animals, by the 8th day *post partum* the weight of the uterine horns had decreased well below the non-pregnant value (Text-figs. 1a and 1b). In the non-lactating animals, hyperinvolution did not occur and, on the 8th day *post partum*, the average weight of the uterine horns from these animals was approximately double that of the lactating group.

The solids content of the uterine horns, expressed on a per cent fat-free basis, was somewhat greater in the non-lactating animals (Text-fig. 1c) although the difference between this group and those allowed to lactate was not marked.

Collagen

Since total uterine hydroxyproline contains only a small percentage (1 to 2%) of non-collagen, i.e. free peptide and elastin hydroxyproline, it may be used to calculate the collagen content without serious error. During the first 24 hr *post partum* there was a lag phase in the rate of loss of collagen (Text-fig. 2a). Thereafter, loss of collagen proceeded very rapidly during the 2nd and 3rd days *post partum* and then decreased. Little difference in the rate of loss of collagen, in lactating and non-lactating animals, was seen until the 3rd day *post partum*.

TEXT-FIG. 3. Mean percentage content of (a) collagen, (b) hexose, (c) hexosamine and (d) uronic acid in uterine horns. ○, Non-lactating; ●, lactating. NP = non-pregnant.

Thereafter, from the 4th to 8th days *post partum*, in the lactating animals, the level of uterine collagen fell below the non-pregnant value while the total collagen in the non-lactating animals still remained above the non-pregnant level. As in the case of the wet and dry weights, at 8 days *post partum* the total uterine collagen was approximately twice as great in the non-lactating as in the lactating rats.

Due to the fact that the rates of loss of weight and collagen were not equal during the post-partum period, the collagen concentration (expressed on a percentage basis) fluctuated considerably (Text-fig. 3a). During the whole of

the period studied the collagen concentration was below the normal value due to the fact that during pregnancy the uterine weight increased by a larger factor (540%) than did the total collagen (350%). The lag in the disappearance of collagen was slightly more pronounced in the non-lactating animals which resulted in a higher value for the uterine collagen concentration in these animals on the 2nd day *post partum* (Text-fig. 3a).

The maximum rate of loss of collagen in both groups occurred on the 2nd and 3rd days *post partum* when the fraction of remaining collagen lost in a 24 hr period was about 1/2 (Text-fig. 2a). It may be seen therefore that, although the initial rate of loss of collagen was less than the overall rate of loss of tissue substance, this difference was reversed on the 2nd and 3rd days *post partum*.

After the 3rd day *post partum*, the rate of loss of collagen decreased in both groups. No significant change occurred between the 5th and 8th days *post partum* in the non-lactating group, whereas in the lactating animals loss of collagen continued, reaching a final value of about half the total non-pregnant amount.

Hexose

The total hexose content of the horns showed an increase at Day 20 of pregnancy of 800% above the non-pregnant level and decreased rapidly after parturition (Text-fig. 2b). In contrast to the collagen curve there was no lag period in the loss of hexose.

The relative increase in total hexose during pregnancy exceeded the increases in either the weight or collagen content of the uterine horns. The relative decrease in hexose content *post partum* was also greater than that of collagen. By the 8th day *post partum* the uterine hexose content in the lactating group had declined to about 1/15 of the value at term, compared with a fall in collagen of 1/7.3 of the initial value. The rate of loss of hexose was similar in both the lactating and non-lactating groups until the 3rd day *post partum* when it decreased sharply in the non-lactating group. The level of hexose in the latter group did not fall below the non-pregnant level and at the 5th and 8th days *post partum* it was about double that of the lactating group.

The concentration of hexose was markedly elevated above the non-pregnant level from the 15th to 20th days of pregnancy in contrast to the collagen concentration which was below the non-pregnant level during this period. After parturition (Text-fig. 3b) there was a steep and sustained fall in hexose concentration, the percentage of hexose in both groups falling below the non-pregnant level; during the period from the 3rd to 8th days *post partum* the hexose percentage was markedly greater in the non-lactating group, although still below the non-pregnant level.

Hexosamine

The total hexosamine content of the uterine horns increased during pregnancy to 490% above the non-pregnant level and decreased sharply after parturition. There was no evidence of a lag period in the post-partum loss of hexosamine. There was also little difference between the lactating and non-lactating rats until the 3rd day *post partum* when the rate of loss of hexosamine

in the non-lactating group decreased sharply while in the lactating animals loss of uterine hexosamine continued and the content fell below the non-pregnant level from the 5th to 8th days *post partum*. The hexosamine content during the first 5 days *post partum* in the lactating group declined to 1/8 of the value at term.

Uronic acid

At term, the total uterine uronic acid was 480% above the non-pregnant value (Text-fig. 2d). Parturition was followed by a rapid loss and the curve showed no evidence of a lag period. As with the other components studied, the results for both lactating and non-lactating rats were very similar until the 3rd day *post partum*, after which the rate of loss of uronic acid decreased in the non-lactating animals at a time when, in the lactating group, the level fell below the non-pregnant level (Text-fig. 2d).

TEXT-FIG. 4. (a) Mean total content and (b) percentage of nucleic acid in rat uterine horns (control, pregnant and post-partum lactating). NP = non-pregnant.

The uterine uronic acid concentration was depressed somewhat below the non-pregnant level during the whole of the period studied although the decrease was not great (Text-fig. 3d). Little difference was observed between the lactating and non-lactating groups.

Nucleic acid

The total nucleic acid content of the uterine horns increased so that at Day 20 of pregnancy the amount was 430% above the non-pregnant level (Text-fig. 4a). However, only a very small increase took place between the 15th and 20th days of pregnancy. The nucleic acid concentration decreased during this period

(Text-fig. 4b) and at term the uterine nucleic acid concentration was well below the non-pregnant value.

A further slight fall in nucleic acid concentration took place during the 1st day *post partum*. From the 2nd day *post partum* onwards a steep rise was found, and the concentration of nucleic acid rose rapidly above the non-pregnant level and was almost doubled by the 8th day *post partum* (Text-fig. 4b). The total nucleic acid content decreased during the involution period (Text-fig. 4a) commencing immediately *post partum* without any obvious lag phase.

TEXT-FIG. 5. Mean values of (a) serum hydroxyproline and (b) serum mucoprotein levels. NP = non-pregnant.

Serum hydroxyproline

Despite the rapid loss of collagen from the uterus only a slight increase was found in the amount of free hydroxyproline in the blood (Text-fig. 5a). The serum hydroxyproline level increased from the non-pregnant level of 3.2 µg/ml to a maximum of 4.8 µg/ml on the 3rd day *post partum*, this rise coinciding with the period of most rapid loss of uterine collagen. The serum hydroxyproline was decreasing on the 5th and 8th days *post partum*.

Serum mucoproteins

During the whole period studied, from the 15th day of pregnancy to the 8th day *post partum*, the serum mucoprotein level was markedly elevated above the non-pregnant level. The maximum level occurred at term and, at this time, it was about 50% above the non-pregnant level. After parturition the level

decreased, showing some evidence of a lag period during the 1st day *post partum* (Text-fig. 5b).

Hormone treatment

Table 1 shows the effect of hormone injections during the first 5 days after labour on the wet weight of the uterus. The mean weight of the horns in the lactating controls on the 8th day *post partum* was approximately half that of the non-lactating animals; the latter did not show hyperinvolution. Hyperinvolution was prevented in the lactating females which were given oestrone and progesterone, although progesterone inhibited lactation after the first day of administration. Oxytocin and prolactin did not induce hyperinvolution in non-lactating animals and did not prevent hyperinvolution in the lactating group. In the non-lactating group the uterine weight was not affected by progesterone whereas oestrone appeared to lower it somewhat although the observation was not statistically significant.

TABLE 1
MEANS AND STANDARD ERRORS OF WET WEIGHTS OF UTERINE HORNS
AT 8 DAYS POST PARTUM

Group sizes are given in parentheses.

	Control	Oestrone	Progesterone	Prolactin	Oxytocin
Lactating	230 ± 45(4)†	448 ± 45(4)**	489 ± 45(4)***	308 ± 64(2)†	276 ± 64(2)†
Non-lactating	431 ± 45(4)	366 ± 45(4)	508 ± 45(4)	548 ± 45(4)	435 ± 52(3)
Non-pregnant	364 ± 41(5)				

† Hyperinvolution.

** Value significantly greater than in control group, $P < 0.01$.

*** Value significantly greater than in control group, $P < 0.001$.

DISCUSSION

OVERALL LOSS OF TISSUE POST PARTUM

The results obtained in this series of experiments showed clearly that the process of post-partum involution resulted in a rapid loss of all the chemical constituents of the uterus which were determined. There was no marked difference in the percentage of fat-free solids in the uteri from lactating and non-lactating rats indicating that the rapid loss in uterine weight *post partum* cannot be ascribed solely to loss of water. Notable differences were recorded in the rates at which various components were resorbed from the uterus. The overall rate of tissue loss during the first 3 days *post partum* appeared to follow a first order law with about one-third of the weight of tissue being resorbed each day (Text-fig. 6a). Since there was little difference in the rates of uterine involution in lactating and non-lactating animals during these first 3 days, the mechanism controlling this initial phase probably differs from that operating later when hyperinvolution occurred but only in lactating animals.

COLLAGEN AND SERUM HYDROXYPROLINE

In contrast to the even rate of overall involution during the first 3 days *post partum*, the rate of loss of collagen was not uniform. An initial lag phase (Harkness & Moralee, 1956) was found during the first 24 hr in both groups followed by a phase of very rapid collagen loss. In view of the varying rate of loss of collagen at different times *post partum* it is not possible to fit the results obtained here to the curve for a first order reaction (Text-fig. 6b). Woessner (1962) found that a linear relationship may be obtained by plotting the square root of the total uterine collagen against time and concluded that collagen was probably being removed from the surface of the fibres. However, the difference in rate of collagen loss to overall tissue loss may be due to the fact that, in the

TEXT-FIG. 6. Logarithms of (a) wet and dry weights and (b) total collagen content of uterine horns. Points lying on straight line correspond to first order reaction. $\times$, Non-lactating; $\bullet$, lactating. NP = non-pregnant.

main, the collagen is distributed extracellularly. It seems possible, therefore, that it is not until dissolution of intracellular tissue protein has proceeded for some time that proteolytic enzymes become available for the breakdown of collagen lying extracellularly. This could account for the lag period in the post-partum resorption of uterine collagen. With regard to the mechanism of tissue breakdown, Woessner (1962) demonstrated that the involuting rat uterus contains a cathepsin, having a pH optimum at 3.5, which at 37° C digests

collagen in homogenates of uterus. According to Woessner, the first stage of collagen breakdown is solubilization of the fibres. The soluble collagen is then thermally denatured at 37° C and digested by acid cathepsins, probably of lysosomal origin. In other experiments (Grant, unpublished) it was found that homogenates of involuting rat uterus did not digest undenatured reconstituted rat tail collagen over a wide range of pH (2 to 10) at room temperature, whereas such preparations were rapidly digested by bacterial collagenase (pH 5 to 9). However, collagenolysis was observed when slices of involuting and non-pregnant uteri were placed on a reconstituted undenatured collagen gel equilibrated with Tyrode solution and maintained at 37° C; this effect has also been recorded by Gross & Lapiere (1962).

As with loss of tissue on a weight basis, the rates of loss of collagen in lactating and non-lactating rats diverged widely after the 3rd day *post partum*. The amount of collagen in the uteri of lactating animals decreased below the non-pregnant level from the 5th day *post partum* onwards, while in non-lactating animals it remained elevated above the non-pregnant control level. It would appear that the level of uterine collagen at a given time is partly dependent on the availability of steroid hormones and that there is a relative deficiency during lactation.

According to Harkness & Moralee (1956) and Woessner (1962) hydroxyproline derived from the breakdown of uterine collagen is removed from the uterus via the blood. In the present study the serum hydroxyproline level was elevated during involution, reaching a peak on the 3rd day *post partum*, that coincided almost exactly with the time of maximum rate of collagen loss from the uterus. However, the magnitude of the rise in serum hydroxyproline was small when compared with the total amount of hydroxyproline lost from the uterus during involution. Since only a small fraction (30%) of the uterine hydroxyproline appears to be excreted in the urine (Woessner, 1962) the present serum hydroxyproline estimations indicate that hydroxyproline mobilized from the involuting uterus is rapidly metabolized.

CARBOHYDRATE

Hexose, hexosamine and uronic acid yielded involution curves similar to those for the wet and dry weights of the uterus, and there was no sign of an initial lag period. The greatest relative change was found in the total hexose content. Most of the hexose probably occurred in the tissue as glycogen and if so, formed the main reserve of available energy of the organ. The energy store was therefore depleted to a very low level in the post-partum period; the loss of hexose followed closely the loss of cytoplasm from the muscle cells which appeared histologically to decrease in size immediately *post partum*.

In contrast to the other components studied, the hexose concentration fell markedly during involution to about half the value at term on the 3rd day *post partum* in both lactating and non-lactating groups. After the 3rd day, however, the hexose concentration was greater in the uteri from non-lactating animals though still well below the level at term. It may be inferred from these findings that the available energy reserve of the uterus is built up to a very high level during pregnancy, possibly to enable the uterus to develop the necessary

contractile force required to expel the foetuses during labour. That a good deal of this available energy is used up during labour is indicated by the very steep fall in the hexose content by the 1st day *post partum* which was relatively larger than was found for any of the other components.

In terms of concentration (percentage) the hexosamine and uronic acid contents showed only slight changes during the latter part of pregnancy and post-partum involution, while the total amounts decreased at about the same rate as the overall loss of tissue. As there was no sign of a lag phase with either of these two substances, it may be inferred that breakdown of mucopolysaccharide commences immediately *post partum*, and that enzymes, capable of breaking down mucopolysaccharides are present in the post-partum uterus in addition to proteolytic enzymes.

SERUM MUCOPROTEIN

It is not possible to correlate loss of uterine mucopolysaccharide components with the level of serum mucoprotein in the same way as the change in serum hydroxyproline level may be correlated with the loss of uterine collagen. It has been found in humans (Shetlar *et al.*, 1950; Gillman & Pillay, 1959) and in rats that the serum mucoprotein level was markedly elevated during the latter part of pregnancy as well as during post-partum involution, the peak occurring at term. The significance of this rise in the serum mucoprotein level is obscure, but Darcy (1957) has shown an increase in the rat's serum mucoprotein (α -glycoprotein) level during periods of rapid tissue growth, e.g. regeneration of the liver, as well as in pregnant animals. The same result was found by Darcy in animals bearing rapidly growing tumours. It therefore appears that the rise in serum mucoprotein here recorded may be related to the overall growth of the uterus and foetuses rather than to the increase and subsequent decrease in the mucopolysaccharide content of the uterus. Darcy (1960) has also found that the mucoprotein is not produced by the growing tissue itself, but arises elsewhere in the body. Our observations fit this hypothesis for, when the uterine mucopolysaccharide content is falling rapidly, the serum mucoprotein is also decreasing. If the serum mucoprotein was derived partly from the uterus it would be expected to show an increase at this time. The serum mucoprotein analyses were restricted to sera from lactating rats only.

NUCLEIC ACIDS

It has been found that growth of uterine muscle during pregnancy is mainly a process of hypertrophy of the cells, the number of cells being almost unaffected (Maibenco, 1960). During involution there is a rapid loss of cytoplasm and at the end of the involution period the nuclei of the muscle cells can be seen to be grouped fairly closely, whereas at term they are widely separated by cytoplasm. In view of this apparent change in the volume ratio of muscle nuclei to cytoplasm, it might be expected that a change in the concentration of nucleic acid would accompany involution since the nucleic acid content of the nucleus is greater than that of cytoplasm. This is clearly demonstrated here (Text-fig. 4b), the concentration of uterine nucleic acid being approximately doubled during

the involution period. Moreover, the fall in the total uterine nucleic acid was less than the relative decreases of all other chemical components estimated during involution.

HORMONE TREATMENT

During the first 3 days *post partum* the rate of involution was similar in both lactating and non-lactating animals. After this time involution practically ceased in the non-lactating rats, but continued to the hyperinvolution stage in the lactating group. This difference has been ascribed to lack of oestrogens (Dawson, 1946) and, moreover, administration of oestrone has been shown to result in an increase in the weight and collagen content of the uterus in the spayed rat (Harkness, Harkness & Moralee, 1957), progesterone having a similar effect. The present results (Table 1) show that both oestrone and progesterone, administered to lactating rats, resulted in significant weight increases compared with the control group. Progesterone, however, inhibited lactation after the first day of administration. The effects of oxytocin and prolactin treatments were not significant. Only the control lactating group showed a significant hyperinvolution ($P < 0.05$) compared with the non-pregnant group.

In conclusion, the fact that uterine involution involved a rapid loss of all the chemical components measured in this study indicates that, in addition to proteolytic enzymes, enzymes capable of splitting mucopolysaccharides, mucoproteins and nucleic acids also appear to be liberated in highly active form immediately following labour. The elucidation of the degradative mechanisms, enzymic or otherwise, operating during the period of post-partum involution of the uterus presents a challenging problem. So far most of the work in this direction has been restricted to proteolytic enzymes and in particular to the breakdown of collagen, but clearly these studies should be extended to the dissolution of the other tissue constituents. It has been suggested that some of these enzymes may be lysosomal in origin (Woessner, 1962) and it may be possible to correlate morphological changes in the subcellular particles of the uterine cells observed by electron microscopy with the release and activation of hydrolytic enzymes during post-partum involution. Moreover, studies of the interactions of various hormones with isolated subcellular fractions from the uterus and isolated uterine enzymes should also yield information which may lead to a clearer understanding of this dramatic biological process.

ACKNOWLEDGMENTS

The author thanks Dr T. Gillman for helpful discussions, Mr P. S. Stewart for skilled technical assistance, and Mr J. G. Rowell for carrying out statistical tests.

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