J. Anat. (1974), **118**, 2, pp. 223–229
With 1 *figure*
Printed in Great Britain

Changes in the thymus, spleen and lymph nodes during pregnancy and lactation in the rat

J. M. McLEAN, J. G. MOSLEY AND A. C. C. GIBBS
Departments of Anatomy and Community Medicine, University of Manchester

(*Accepted* 30 *April* 1974)

INTRODUCTION

The enigmatic success of the fetal allograft, discussed in a number of recent reviews (Billingham, 1964; Kirby, 1968; Anderson, 1971; Beer & Billingham, 1972), may depend on several immunological factors. Many of the effects of immunological challenge and response are seen in the reticulo-endothelial system and several workers have reported changes in the reticulo-endothelial system of the maternal animal during pregnancy.

An increase in iliac lymph node weight during pregnancy has been observed in rats (Hellman, 1930; Gregoire, 1947; Beer & Billingham, 1972) associated with multiplication of large pyroninophilic and plasma cells in rats (Shivatcheva, 1972). Observations have also been made of thymic involution during pregnancy (Persike, 1940) persisting during lactation and associated with similar changes in the spleen (Gregoire, 1947).

The present work was designed to confirm these observations and to determine their nature in more detail.

METHODS

Four groups of female animals were used, each group consisting of 10 sexually mature Sprague Dawley rats taken from a closed colony. Group 1 animals were virgin controls and Groups 2, 3 and 4, having been mated with 4 related Wistar males to minimize genetic disparity among the conceptuses, were killed 10, 20 and 40 days post-coitum respectively. Group 4 animals were allowed to suckle their young.

Animals were killed with excess ether anaesthesia and the thymus, spleen, popliteal and iliac lymph nodes and uterus were removed and weighed. The iliac lymph nodes, two to four in number, drain the uterus (Tilney, 1971). Weighed portions of the lymph nodes, thymus and spleen were teased out in Eagle's medium to prepare cell suspensions which were then washed and re-suspended in fresh medium. White cell counts were made with a haemocytometer. Smears were prepared, after appropriate dilution of the cell suspensions with Eagle's medium, using 0·1 ml aliquots in a Shandon–Elliott cytocentrifuge. The smears were dried in air and stained with Leishman (Darmady & Davenport, 1963). A permanent preparation was made with a suitable mounting medium and the differential white cell counts performed by inspection of 150–200 cells, or 10 fields if the cell density of the smear was low. Only

Table 1. *Mean tissue weight (g/100 g body weight)*

Group	Thymus	Spleen	Popliteal nodes	Iliac nodes
1	0·161	0·277	0·0046	0·0098
2	0·164	0·278	0·0055	0·0173
3	0·119	0·260	0·0045	0·0239
4	0·059	0·186	0·0038	0·0080
Least significant difference	0·030	0·038	0·0018	0·0066

lymphoblasts and large and small lymphocytes were counted on each slide. The lymphoblast was recognized as a cell with a coarse nucleus, nucleoli and light blue cytoplasm free of granules, and a large lymphocyte as a cell with an homogeneous nucleus and a rim of light blue cytoplasm. The sum of lymphoblasts and large lymphocytes was expressed as a percentage of the total number of cells counted in the smear.

RESULTS

The mean tissue weight, mean white cell count, and mean differential white cell count for popliteal lymph nodes in Group 1 are based upon 8 specimens only, since these nodes in 2 of the virgin animals were unavailable. In addition, differential white cell counts were not obtained for one thymus and one popliteal node in Group 3 because these two smears were inadequate.

For each site, the tissue weights, white cell counts and differential white cell counts were subjected to an analysis of variance, missing values being dealt with according to the method of Yates (1933), to determine whether there were significant differences between the groups. Least significant differences were calculated for each parameter at each site to establish which differences between groups were significant. In the tables and the figure the value given for the least significant difference is that appropriate for comparison with the group with fewest results and this is the minimum difference between the means of any two groups which is significant at the 5% level.

Tissue weights. The iliac lymph nodes from each rat were weighed together, as were the popliteal nodes, giving total iliac and popliteal node weights. Thymus, spleen, iliac and popliteal node weights were expressed per 100 g of body weight, where this was the weight of the animal minus the weight of the uterus and its contents. These results are shown in Fig. 1 and Table 1.

The thymus showed a significant loss of weight in late pregnancy and a further significant loss after delivery and during lactation. A significant reduction in the weight of the spleen also occurred during lactation.

Iliac lymph node weight rose significantly in early pregnancy and again in late pregnancy but returned to virgin levels after delivery. No significant weight changes occurred in the popliteal nodes.

White cell counts. The white cell counts per 100 mg of tissue (Fig. 1 and Table 2) are a measure of the white cell density of the tissue.

Table 2. *Mean white cell count* ($\times 10^6$/100 *mg tissue*)

Group	Thymus	Spleen	Popliteal nodes	Iliac nodes
1	90·2	37·1	34·9	61·6
2	150·4	71·3	23·4	42·3
3	113·6	47·0	36·3	73·3
4	91·4	50·1	18·1	30·0
Least significant difference	32·42	14·63	13·76	17·88

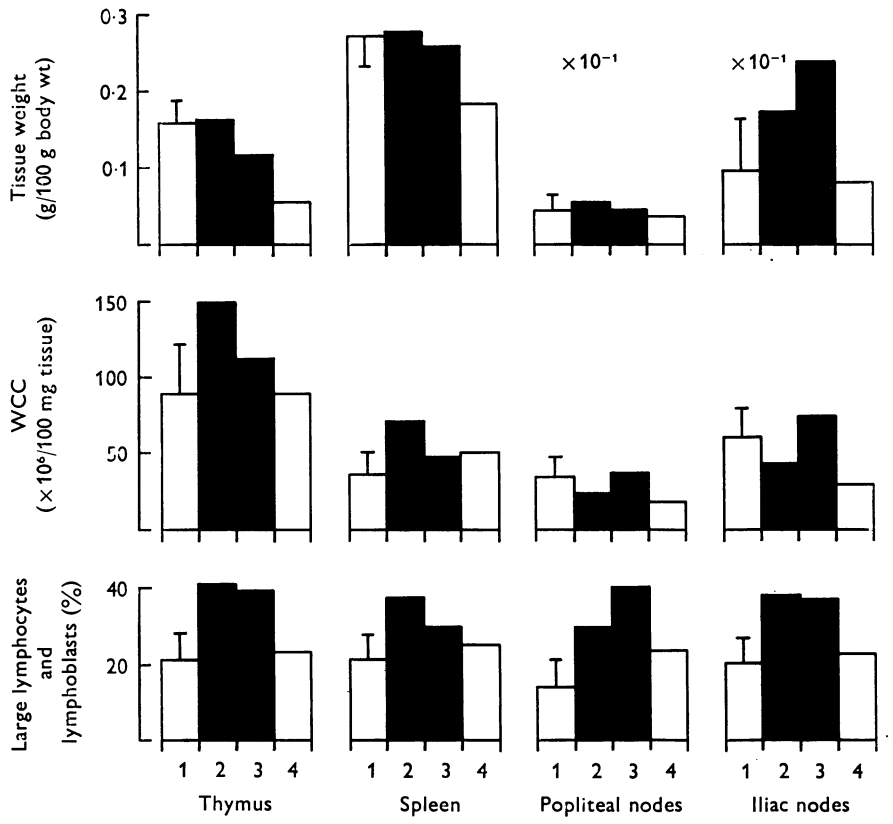

Fig. 1. Tissue weights, white cell counts and differential white cell counts of thymus, spleen, popliteal and iliac lymph nodes. Group 1 consists of virgin animals, Groups 2, 3 and 4 are 10, 20 and 40 days post-coitum respectively. The vertical line on each Group 1 histogram is the least significant difference for those 4 groups and is the minimum difference between the means of any two groups which is significant at the 5% level.

Table 3. *Mean percentage of lymphoblasts and large lymphocytes in the differential white cell count of each tissue*

Group	Thymus	Spleen	Popliteal nodes	Iliac nodes
1	21·3	21·1	14·0	20·0
2	41·1	37·8	29·2	37·6
3	39·8	29·8	39·9	36·9
4	23·4	25·0	23·3	22·2
Least significant difference	6·8	6·6	7·2	6·6

The cell density of the thymus in early pregnancy was significantly greater than that in virgin, near-term and delivered animals. A similar pattern of changes in white cell density occurred in the spleen. The iliac lymph nodes in early pregnancy and after delivery were significantly less cellular than those of late pregnancy and virgin animals. The popliteal node cell density was significantly less in the delivered group than in the virgin and near-term animals.

Differential white cell counts. The differential white cell counts (Fig. 1 and Table 3) are expressed as the percentage of lymphoblasts and large lymphocytes in the total of lymphoblasts and large and small lymphocytes counted in the smear.

In the virgin control animals the mean differential white cell counts for thymus, spleen and iliac lymph nodes were very close together and although the mean for the popliteal nodes seemed low it was not significantly different from the other three.

A significant increase in the proportion of large lymphocytes and lymphoblasts occurred in all tissues during pregnancy with a return to control virgin levels after delivery except in the popliteal nodes where the proportion remained significantly higher than in the virgin group.

DISCUSSION

Thymus

The central role of the thymus in the development of immunological competence is firmly established. Its role in the maintenance of immunological competence, however, is not so clear since claims that adult thymectomy influences immunological capacity (Miller, 1962) and prolongs partial tolerance (Claman & Talmage, 1963) are countered by others suggesting that it is without significant effect (Hayward & Soothill, 1973).

The changes observed in the thymus during pregnancy and lactation are shown in Fig. 1. While thymic weight remains unchanged in early pregnancy significant increases occur in cell density and in the proportion of large lymphocytes and lymphoblasts in the differential white cell count. These changes suggest mobilization and differentiation of structural supporting cells to join the white cell series in response to some specific stimulus. Near term the tissue weight and cell density fall, producing an absolute reduction of thymic tissue, but the proportion of large

lymphocytes and lymphoblasts in the differential white cell count remains elevated. Presumably the stimulus to cell proliferation and differentiation persists and there is an increased outflow of cells to the peripheral lymphoid organs. After delivery and while lactation continues, thymic weight loss is progressive but the cell density and differential white cell count have returned to virgin levels, suggesting that any specific stimulus has ceased.

Thymic weight loss during pregnancy and lactation was expected since it has been observed previously (Persike, 1940; Gregoire, 1947) but the increased cellular activity observed only during pregnancy is paradoxical unless its cause is unrelated to that of involution. It is generally assumed that thymic regression during pregnancy and lactation is produced by hormonal factors since administration of oestrogen, testosterone, ACTH or cortisone has this effect. There is no evidence, however, to suggest that these hormones stimulate cellular activity in the thymus (Clark, 1973).

Spleen

The spleen, although playing a major role in erythrocyte destruction, also stores and produces erythrocytes, platelets, granulocytes and lymphocytes. It is also the major antibody producing tissue following challenge by blood borne antigen (Humphrey & White, 1970).

The changes observed in the spleen during pregnancy and lactation are shown in Fig. 1. With the exception of weight loss, which is significant only during lactation, the pattern of change is identical with that of the thymus. Splenic involution during lactation has been reported (Gregoire, 1947) and was thought to be due to hormonal factors. The increase in the proportion of large lymphocytes and lymphoblasts in the splenic differential white cell count observed during pregnancy is similar to that observed following subcutaneous and intraperitoneal challenge of rats with equine gamma globulin (McLean, 1972).

Lymph nodes

Lymph nodes filter lymph, phagocytose particulate material, synthesize antibody and participate in the proliferation and re-circulation of lymphocytes.

An increase in the weight of iliac lymph nodes during pregnancy has been observed in the rat (Hellman, 1930; Gregoire, 1947) and rabbit (McLean & Allen, unpublished). Beer & Billingham (1972) noted striking enlargement of the rat para-aortic nodes during inter-strain, but not intra-strain pregnancy.

The changes observed in the iliac nodes during pregnancy and lactation are shown in Fig. 1. The significant increase in lymph node weight of early pregnancy coincides with a significant fall in cell density. Comparison of the total white cell counts of these lymph nodes in the virgin and early pregnancy groups showed no significant difference between them, suggesting that weight gain in early pregnancy is due to increased fluid content, a possible event even in intra-strain pregnancy. The further significant weight gain of late pregnancy in association with virgin cell density levels indicates hyperplasia in the regional lymph nodes. During lactation the lymph node weight returns to the control level but cell density falls below it, suggesting an increase in fluid content over that of the virgin state.

There is a significant increase in the proportion of large lymphocytes and lympho-

blasts in the differential white cell count throughout pregnancy, returning to control virgin levels after delivery. Statistical examination of the differential white cell counts of thymus, spleen, iliac and popliteal nodes shows no significant difference between any of the sites in virgin or delivered animals, and no significant difference between thymus, spleen and iliac nodes in early pregnancy. This observation suggests a specific stimulus affecting thymus, spleen and iliac nodes equally at this stage of pregnancy. Studies earlier in pregnancy might indicate which tissue, if any, first shows this increase of lymphoblasts and large lymphocytes in its differential white cell count.

Gregoire (1947) observed involution of distal lymph nodes during and after pregnancy in the rat but this was not confirmed in the rabbit (McLean & Allen, unpublished). The changes in the popliteal lymph nodes during pregnancy and lactation are shown in Fig. 1. There was no significant change in the weight of popliteal lymph nodes, although their cell density was significantly reduced, as was that of the iliac lymph nodes, during lactation. The pattern of change in popliteal node differential white cell counts suggests that the stimulus to large lymphocyte and lymphoblast production increases during pregnancy and remains after delivery. However the stimulus to large lymphocyte and lymphoblast production in thymus, spleen and iliac nodes during pregnancy appears to cease after delivery. This apparently anomalous behaviour of popliteal nodes is unexplained.

These results demonstrate significant changes in the maternal reticulo-endothelial system during pregnancy in the rat. Some of these changes are recognizably part of the host's immunological response to antigenic challenge and, presumably, in this case are due to the presence of the allogenic products of conception. Since pregnancy usually proceeds successfully to term, it is clear that despite initiation of the maternal immunological response in both central and peripheral lymphoid tissues, it is somehow not completed. All explanations of the success of homografts (allografts) are excusatory in character, i.e. they are explanations of why the homograft (allograft) failed to fail (Medawar, 1957). It has been suggested that the success of pregnancy is due to impaired T cell activity (Finn, Hill, Govan, Ralfs, Gurney & Denye, 1972; Putilo, Hallgreen & Yunis, 1972) or to the presence of blocking antibodies (Leikin, 1972; Curzen Jones & Gangas, 1972). Our findings are compatible with either explanation.

SUMMARY

Changes in weight, cell density and differential white cell count of the thymus, spleen, iliac and popliteal lymph nodes of virgin, pregnant and lactating rats were examined.

Significant weight loss occurred in the thymus during late pregnancy and lactation and in the spleen during lactation, while significant weight gain occurred in the iliac lymph nodes progressively during pregnancy.

The cell density of thymus and spleen rose significantly during early pregnancy and that of the iliac lymph nodes fell significantly during early pregnancy and lactation.

The proportion of large lymphocytes and lymphoblasts in the differential white cell counts of thymus, spleen, iliac and popliteal lymph nodes increased significantly during pregnancy.

These findings are discussed in relation to the maternal immunological response to pregnancy.

We would like to thank Professor G. A. G. Mitchell for his encouragement in this work, Dr R. G. W. Ollerenshaw for preparing the histograms, and Miss K. E. Latchem for typing the manuscript.

REFERENCES

ANDERSON, J. M. (1971). Transplantation – nature's success. *Lancet* **2**, 1077–1082.

BEER, A. E. & BILLINGHAM, R. E. (1972). Immunobiology of mammalian reproduction. *Advances in Immunology* **14**, 1–84.

BILLINGHAM, R. E. (1964). Transplantation immunity and the maternal–fetal relation. *New England Journal of Medicine* **270**, 667–672 and 720–725.

CLAMAN, H. N. & TALMAGE, D. W. (1963). Thymectomy: prolongation of immunological tolerance in the adult mouse. *Science* **141**, 1193–1194.

CLARK, S. L. Jr. (1973). The intrathymic environment. *Contemporary Topics in Immunobiology*. **2**. *Thymus Dependency*. (Eds. A. J. S. Davies & R. L. Carter) 77–99. New York and London: Plenum Press.

CURZEN, P., JONES, E. & GANGAS, J. (1972). Immunological responses in pregnancy. *British Medical Journal* **4**, 361.

DARMADY, E. M. & DAVENPORT, S. G. T. (1963). *Haematological Technique*. London: J. and A. Churchill Ltd.

FINN, R., HILL, C. A. St., GOVAN, A. J., RALFS, I. G., GURNEY, F. J. & DENYE, V. (1972). Immunological responses in pregnancy and survival of fetal homograft. *British Medical Journal* **3**, 150–152.

GREGOIRE, C. H. (1947). Factors involved in maintaining involution of the thymus during suckling. *Journal of Endocrinology* **5**, 68–87.

HAYWARD, A. R. & SOOTHILL, J. F. (1973). Some clinical implications of thymus dependent functions. *Contemporary Topics in Immunobiology*. **2**. *Thymus Dependency*. (Eds. A. J. S. Davies and R. L. Carter), 351–361. New York and London: Plenum Press.

HELLMAN, T. (1930). Quoted by GREGOIRE, C. H. (1947).

HUMPHREY, J. H. & WHITE, R. G. (1970). *Immunology for Students of Medicine*. 3rd ed. Oxford: Blackwell.

KIRBY, D. R. S. (1968). In *Advances in Reproductive Physiology* **3**. (Ed. A. McLaren), 33–79. London: Logos Press.

LEIKIN, S. (1972). Depressed maternal lymphocyte response to phylohaemagglutinin in pregnancy. *Lancet* **2**, 43.

MCLEAN, J. M. (1972). The cellular response in rats to challenge with horse gamma globulin. *Journal of Anatomy* **111**, 477.

MEDAWAR, P. B. (1957). The immunology of transplantation. *The Harvey Lectures 1956–57*, 144–176.

MILLER, J. F. A. P. (1962). Immunological significance of the thymus of the adult mouse. *Nature* **195**, 1318–1319.

PERSIKE, E. C. (1940). Involution of the thymus during pregnancy in young mice. *Proceedings of the Society of Experimental Biology and Medicine* **45**, 315–317.

PUTILO, D. T., HALLGREEN, H. M. & YUNIS, E. D. (1972). Depressed maternal lymphocyte response to phytohaemagglutinin in human pregnancy. *Lancet* **1**, 769–770.

SHIVATCHEVA, T. (1972). Research on the cell reaction of regional lymph nodes of the rat uterus during pregnancy. *Proceedings of the XVth Morphological Congress, BRNO*, p. 202.

TILNEY, N. L. (1971). Patterns of lymphatic drainage in the adult laboratory rat. *Journal of Anatomy* **109**, 369–383.

YATES, F. (1933). The analysis of replicated experiments when the field results are incomplete. *Empire Journal of Experimental Agriculture* **1**, 129–142.