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A HISTOLOGICAL STUDY OF THE EFFECT OF CORTISOL AND SOME SEX STEROIDS ON THE IMMUNE RESPONSE TO SHEEP ERYTHROCYTES BY THE MOUSE

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ABSTRACT

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Sections of the spleen, thymus, lymph nodes and liver, collected from mice at various time intervals after injection with either steroid hormones only, sheep red blood cells (SRBC) only, or SRBC plus steroid hormones, were compared by histological examination.

A regimen of 3 injections of 4 mg of cortisol given at 24 hourly intervals was shown to have a more severe effect than 3 injections of 1 mg given at the same times irrespective of whether SRBC were injected or not. The thymic cortex showed rapid and extensive depletion of lymphocytes very soon after corticosteroid treatment and did not recover until about the 8th day. The medulla was affected to a lesser extent. Spleens and lymph nodes showed early lymphocyte destruction, active ingestion of debris by macrophages, and germinal centres were considerably decreased in number and less clearly demarcated in corticosteroid-treated animals than in SRBC immunized controls.

Spleens and lymph nodes of mice that received SRBC only exhibited the characteristic morphology of active germinal centre development associated with the immune response.

Corticosteroid treatment of mice sensitized with SRBC caused an increase in neutrophilic promyelocytes in bone marrow smears to the 4th day, whereafter their numbers returned to normal.

The normoblasts were decreased on the 2nd and 3rd days whereafter they increased to normal. Plasma cells were increased in SRBC injected animals in bone marrow smears.

Of the effects of the sex steroids studied the most notable was a drastic effect of estradiol on the thymus; both the cortex and medulla were completely depleted of lymphocytes and could hardly be distinguished.

INTRODUCTION

It is now well established that corticosteroids cause involution of lymphatic tissue in varying degrees proportional to the dosage and nature of the steroid (Dougherty, 1952; Santisteban & Dougherty, 1954; Ishidate & Metcalf, 1963; Dougherty, Berliner, Schneebeli & Berliner, 1964) and various publications have appeared dealing with morphological changes in lymphoid tissue caused by cortisol and its analogues with concomitant immunization (Bjørnboe, Fischel & Stoerck, 1951; Craig, 1952; Wissler, Fitch, La Via & Gunderson, 1957) or without it (Dougherty & White, 1945; Ringertz, Fagreus & Berglund, 1952; Winblad & Johnson, 1952; Ishidate & Metcalf, 1963; Dougherty *et al.*, 1964). However, the picture is incomplete in that either only 1 or 2 organs were studied or, in some cases, dose regimens were used which did not lead to a drop in antibody production.

In a preceding paper (Hellig & Waldek, 1974) a significant reduction in plaque-forming cells (PFCs) was described with a dose regimen of 3 injections of 4 mg cortisol, given at 24 hourly intervals to mice measuring 20 g. The effect was maximal when the 1st injection was given 6–8 h before the immunizing dose. Accordingly a complete chronological study has now been made of the histological changes in all lymphoid organs and the liver using this dose regimen with and without concomitant immunization. For clarity, a study of sheep red blood cell (SRBC)-injected mice, without steroid treatment, is included.

In order to obtain a wider comparison the above regimen was experimentally compared with the effects produced by 3 doses of 1 mg cortisol, and by the non-glucocorticoid sex steroids progesterone, testosterone and estradiol. The latter have been reported also to exert some effects on lymphatic tissue (Dougherty, 1952).

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Appropriate controls were included to evaluate the possible effects of the suspending media.

MATERIALS AND METHODS

Experimental animals

Eight-week-old white female mice obtained from the Onderstepoort closed colony and measuring up to 20,5 ±1,0 g were used in all the experiments. They were raised and kept under conventional conditions and fed a balanced ration.

SRBC

The immunizing dose consisted of 0,2 ml 10% v/v SRBC in saline, prepared as described previously (Hellig & Waldek, 1974). The injection was given intravenously (i.v.).

Steroid preparations

The sources, doses and suspension or solution vehicles are given in Table 1.

Steroid treatment

Aliquots (0,2 ml) of a solution of each steroid were injected intraperitoneally (i.p.). The 1st injection of any steroid was given i.p. in the designated dosage on Day 0 at 08h00, prior to SRBC immunization at 16h00 of the same day. Thereafter 2 more injections of steroid were given on Day 1 and Day 2 at 08h00.

Schedule of experiments

Two major experiments were performed. In the 1st experiment all animals (including the controls) were immunized with SRBC. The test animals were previously injected i.p. with glucocorticoids or sex steroids in aqueous solution or oil suspension according to the dosages given in Table 1. Control mice were injected with suspending medium alone, except that physiological saline was used instead of water. Tissues for histological studies were collected (see below) from animals sacrificed at 2 time intervals: (1) 24 h after SRBC injection, i.e. 32 h after administration of either the steroid or suspending agent alone, and (2) Day 5 after SRBC injection.

TABLE 1 Steroids injected 3 times at 24 hourly intervals, starting 6–8 h before the immunizing dosage of SRBC

Steroid	Dose	Physical state	Suspended in	Dissolved in
Cortisol [a]	1,0 mg or 4,0 mg	Suspension	Water or Ethanol: almond oil 1:4*	
Progesterone [b]	2,0 mg	Solution		Almond oil
Testosterone propionate, BP [c]	2,0 mg	Solution		Ethyl oleate: arachis oil 2:5 and diluted with almond oil[d]
Estradiol [a]	450–500 µg	Suspension	Water	
Efcortolan [e]	2 mg	Solution		Buffer [f]
Betsolan [e]	0,2 mg or 1,0 mg	Solution		Buffer [f]

* Oil suspension

(a) Merck
(b) Evans Medical Ltd.
(c) British Drug Houses
(d) Supplied as a gift from Haarman & Reimer (Pty) Ltd.
(e) Glaxo Laboratories. Efcortolan is a soluble preparation of cortisol sodium succinate and Betsolan is a soluble cortisol analogue
(f) As supplied by the Glaxo Laboratories

The 2nd experiment was a time study in which the mice were divided into 3 groups. Group A received 3 doses of 4 mg cortisol in oil suspension i.p. at 24 hourly intervals. Group B received the same dose of steroid plus 0,2 ml 10% v/v SRBC 8 h after the 1st steroid injection, whereas Group C received only SRBC at the same time as Group B. Tissues for histological studies were collected 4, 8 and 12 h after the 1st steroid injection and thereafter daily at 08h00 and 16h00 for 8 consecutive days as outlined below. Routinely only 1 animal was examined at each time period for each dose regimen.

Histological preparations

Mice were killed by cervical dislocation. Their spleens, livers and thymuses were immediately excised and fixed in 10% formalin. The popliteal lymph nodes were included in some instances. Paraffin sections were then cut at 4 µm in duplicate and stained with haematoxylin and eosin. Bone marrow smears were prepared from the ribs, fixed in methanol and stained with freshly prepared 10% aqueous Giemsa solution (pH 6,9). The different smears were compared microscopically on a semi-quantitative basis.

Results and Discussion

The effect of different steroids and suspension media

Hellig & Waldek (1974) demonstrated that the spleens of SRBC-injected mice showed the maximum immunological response at Day 4–5 after sensitization. A few PFCs could first be detected at 24 h (Oellermann, personal communication, 1974). Accordingly, these 2 times were chosen for the first experiment. The steroids were injected in the doses given in Table 1 utilizing the regimen which resulted in the greatest diminution of PFC production when 3 doses of 4 mg cortisol were used (Hellig & Waldek, 1974).

Apart from the primary aim of establishing the action of cortisol and its analogues, the effects of progesterone, testosterone and estradiol were also studied, as very little has been published on the effect of sex steroids on lymphopoietic tissue (Dougherty, 1952).

It is emphasized that all mice in this 1st experiment were injected with SRBC, thus the effects of the steroids are superimposed on the prevailing immune response. The mice were examined at 24 h and 5 days post-vaccination.

The following histological changes were observed in the various organs.

Liver

1. Mice injected with SRBC (alone or with saline gave similar results): slight vacuolization was observed around the central veins of the lobule (Fig. 1).
2. Mice injected with SRBC plus oil: Very fine vacuolization resulted throughout the liver lobules, the small vacuoles being almost perfectly round with well defined margins probably due to ingestion and metabolization of the oil (Fig. 2).
3. Mice injected with SRBC plus either 1 or 4 mg doses of cortisol in either water or oil: Large irregular vacuoles appeared in the liver cells throughout the lobule with the 1 mg dose (Fig. 3). This effect was acerbated with the 4 mg dose, especially at the 24 h time period when some liver cell nuclei were completely surrounded by large vacuoles (Fig. 4). At Day 5 the vacuoles had decreased in size, but were still apparent throughout the lobules.
4. Mice injected with SRBC plus 0,2 or 1 mg Betsolan: The effects were very similar to those produced by 4 mg cortisol, though the excessive early vacuolization persisted more intensely at Day 5 than with cortisol.

The vacuoles produced by the corticosteroids were irregular and not as well defined as those produced by oil alone and are most probably due to accumulation of glycogen; this phenomenon is therefore described as glycogen vacuolization. A similar effect of cortisol on the liver has been described (Dougherty & White, 1945).

The thymus

1. Mice injected with SRBC (alone or with saline): Both the cortex and the medulla were well developed and clearly defined. The nuclei did indeed look slightly pyknotic, but no cellular breakdown or phagocytosis was observed (Fig. 5).
2. Mice injected with SRBC plus oil: Although the cortex and medulla remained well developed and clearly demarcated, the lymphocyte nuclei were severely pyknotic and considerable phagocytosis was observed with concomitant depletion of lymphocytes and thinning of the cortex at 24 h (Fig. 6). No phagocytosis could be observed in the cortex by Day 5.

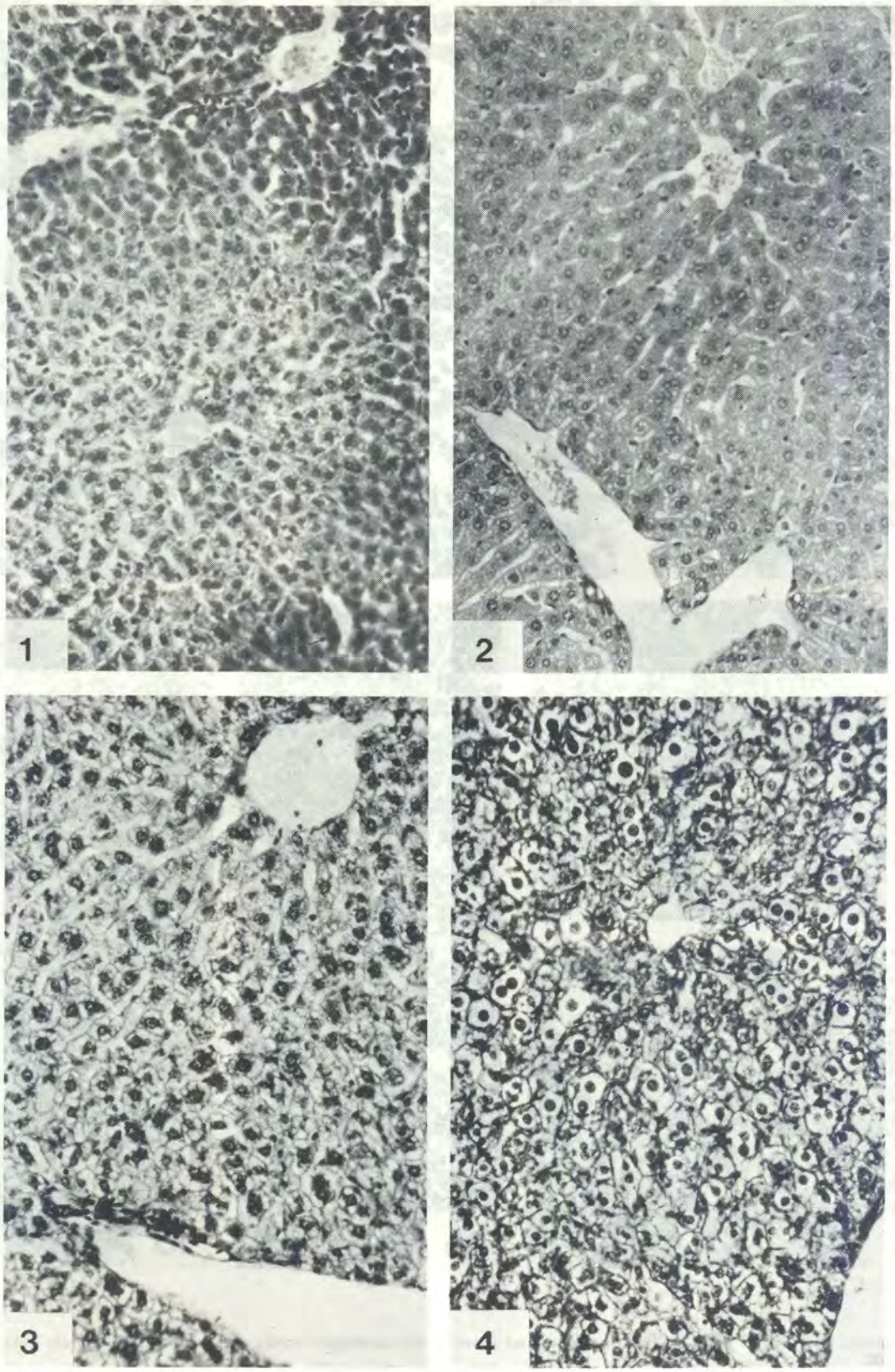


FIG. 1 Liver; Day 5 after SRBC (alone or plus saline) revealing vacuolization around central vein only. ×200

FIG. 2 Liver; Day 5 after SRBC plus oil revealing fine vacuolization throughout the liver lobule, probably due to ingestion of oil. ×200

FIG. 3 Liver: Day 5 after SRBC plus 1 mg doses of cortisol suspended in either water or oil, revealing large vacuoles throughout the lobules. ×200

FIG. 4 Liver: 24 h after SRBC plus 4 mg doses of cortisol revealing large vacuoles completely surrounding the nuclei. ×200

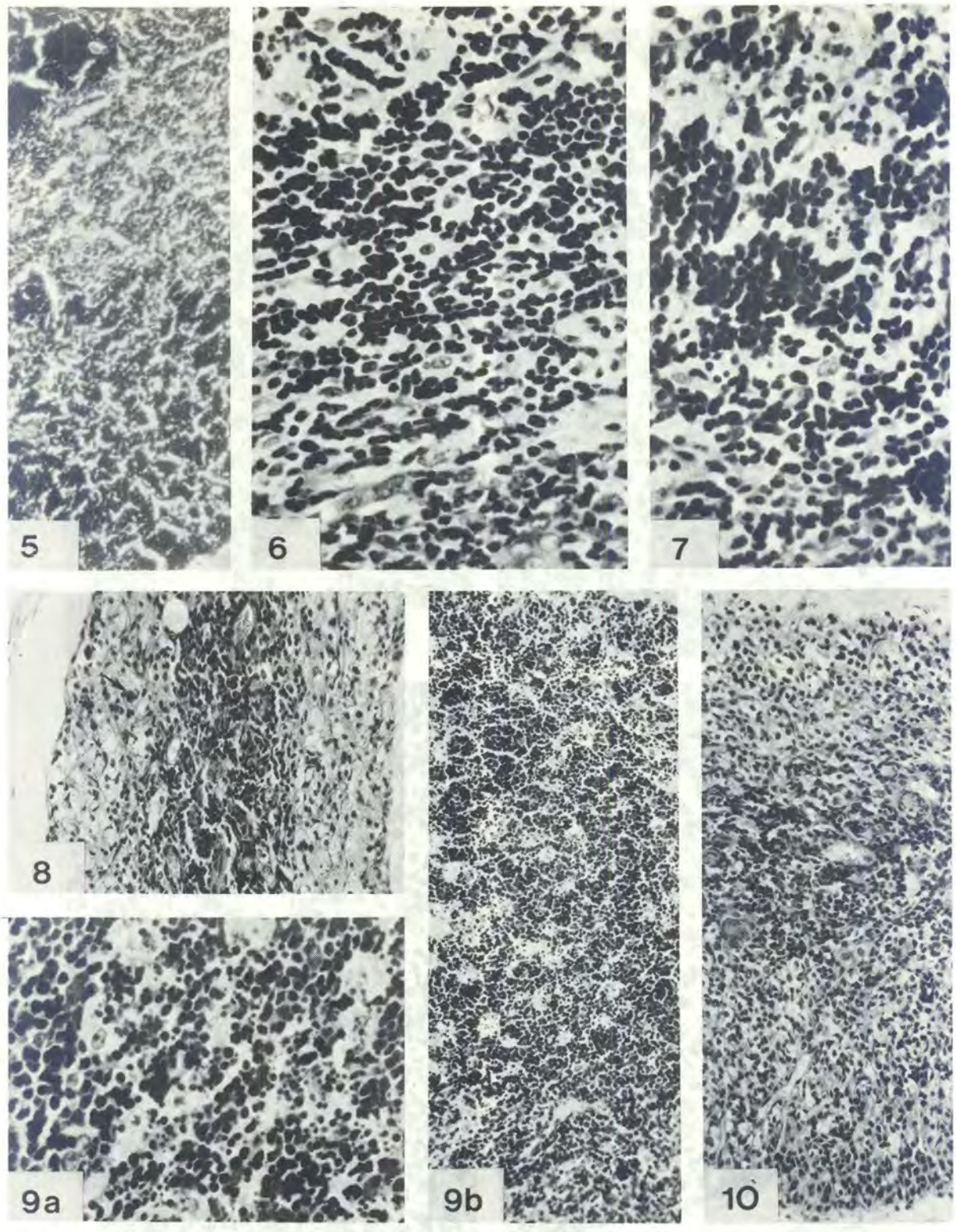


FIG. 5 Thymus: Day 5 after SRBC (alone or plus saline) shows a well developed cortex and medulla, but slightly pyknotic nuclei, ×200

FIG. 6 Thymus: 24 h after SRBC plus oil suspending agent revealing pyknotic nuclei, considerable phagocytosis and concomitant depletion of lymphocytes and thinning of the cortex. ×500

FIG. 7 Thymus: 24 h after SRBC plus 1 mg doses of cortisol in oil suspending medium revealing more severe phagocytosis in cortex with resultant thinning of cortex in certain areas. ×500

FIG. 8 Thymus: Day 5 after SRBC plus 4 mg doses of cortisol revealing nuclear debris following severe cortical necrosis and phagocytosis. The nuclear debris can be seen as aggregated pyknotic bodies (arrow); macrophages with vacuolated stellate cytoplasm bear testimony to phagocytosed nuclear debris which has been completely digested. ×200

FIG. 9 Thymus: 24 h after SRBC plus 4 mg doses of cortisol in aqueous suspension showing the most severe degree of karyorrhexis and phagocytosis of lymphocytes seen with corticosteroid. Fig. 9a ×500, Fig. 9b ×200

FIG. 10 Thymus: Day 5 after SRBC plus 4 mg doses of cortisol in aqueous suspension. The cortex is completely depleted of lymphocytes and phagocytosis has ceased. Macrophages are resuming normal appearance although vacuolisation can still be seen

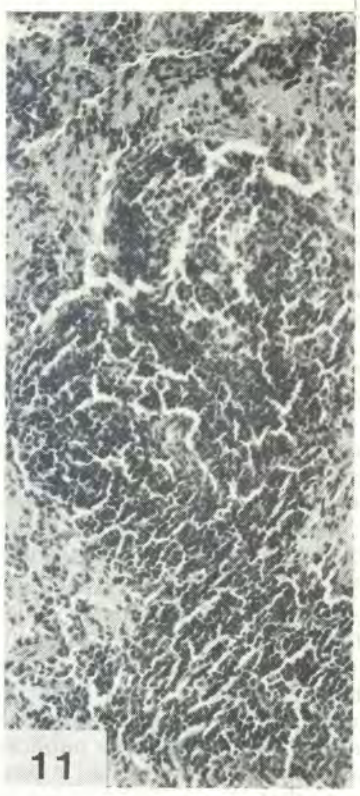
3. Mice injected with SRBC plus 1 or 4 mg cortisol in oil: At 24 h, with the 1 mg dose, there was very severe phagocytosis in the cortex, though this phenomenon was only occasionally seen in the medulla. Although well demarcated, the cortex was rather thin in certain areas (Fig. 7); thus considerable depletion of lymphocytes at this stage could be inferred. By Day 5 phagocytosis could no longer be observed in the cortex, although the nuclei appeared more pyknotic than in the medulla and were less tightly packed. The 4 mg dosages affected the thymus similarly, but more severely. Peripheral cortical phagocytosis of nuclear debris could still be observed, but the nuclear debris tended to aggregate within the macrophages forming globular pyknotic bodies which, after complete solution, left large macrophages with vacuolated stellate cytoplasm (Fig. 8).
4. Mice injected with SRBC plus 1 or 4 mg cortisol in aqueous suspension: No significant difference was observed between the 2 doses. At 24 h this treatment caused the most excessive and severe degree of karyorrhexis and phagocytosis of nuclei yet seen (Fig. 9a & b). The nuclei in the cortex were extremely pyknotic and fragmented. The medulla was much less affected, and only a few macrophages with cellular debris could be seen here. By Day 5 active phagocytosis could no longer be observed; the macrophages were still vacuolated but contained no cellular debris. Some lymphocytes were retained in the medulla but the cortex was largely depleted (Fig. 10). These effects of cortisol in aqueous medium proved conclusively that it was not the oil suspending medium alone which produced these major changes.
5. Mice injected with SRBC plus 0,2 mg Betsolan: Although the histological picture was the same as the above at 24 h, the thymus cortex revealed fewer pyknotic nuclei by Day 5 as most were more vesicular. Relatively few macrophages were seen, and these had small vacuoles which contained an occasional particle of debris in their cytoplasm. Thus the thymus appeared to recover within 5 days from the effects of the synthetic steroid, although cortisol itself produced a more drastic and prolonged effect.
6. Mice injected with SRBC plus Efcortolan: By Day 5 only a few lymphocytes were retained in the medulla, the cortex being completely denuded of lymphocytes (Fig. 14) and containing macrophages filled with yellowish granules, as seen after HE staining. Hassal's corpuscles were barely visible while the epithelial network was contracted.
7. Mice injected with SRBC plus progesterone: At Day 5 there was lymphocytic depletion of the thymic cortex, but very little phagocytosis was seen and no pyknosis or oedema. The epithelial reticular network was contracted, but the medulla appeared to be normal or slightly denser than normal, suggesting that the cortical lymphopenia was due to migration from the cortex to the medulla.
8. Mice injected with SRBC plus testosterone propionate: In contrast to progesterone, injection of this steroid resulted in a greater accumulation of lymphocytes in the cortex, with a normal medulla.
9. Mice injected with estradiol: Treatment with this steroid resulted in a thymus which could hardly be recognized as such. In addition to no definite cortex and medulla being present at Day 5, the nuclei were all very pyknotic (Fig. 15).

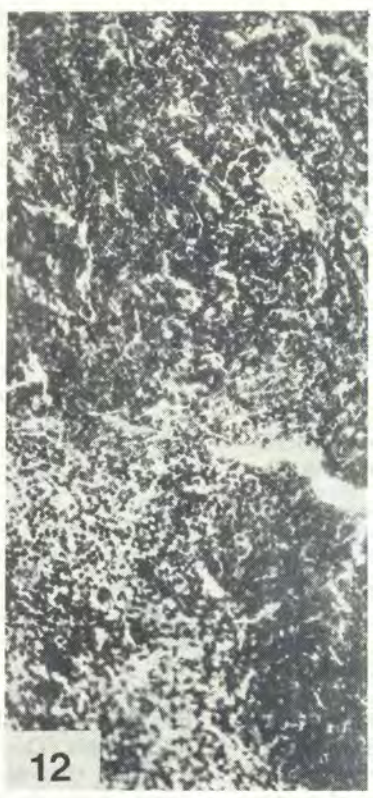
The effects of corticosteroids on the thymus reported here, especially the well-nigh total depletion of cortical lymphocytes, are in accord with previous work (Ringertz *et al.*, 1952; Ishidate & Metcalf, 1963). Although the thymuses were not mass measured, cortisol-treated thymuses were observed to be greatly reduced in size by 24 h and remained small up to Day 5. This is in agreement with measurements of thymic mass after corticoid treatment (Santisteban & Dougherty, 1954; Ishidate & Metcalf, 1963) which revealed that the thymus decreases in size within a few hours, and takes considerably longer to regenerate than does the spleen. It was impossible to conclude from the light microscopic observations whether endodermally or mesodermally derived reticular cells were responsible for the active phagocytic activity revealed by the thymic cortex after corticoid treatment.

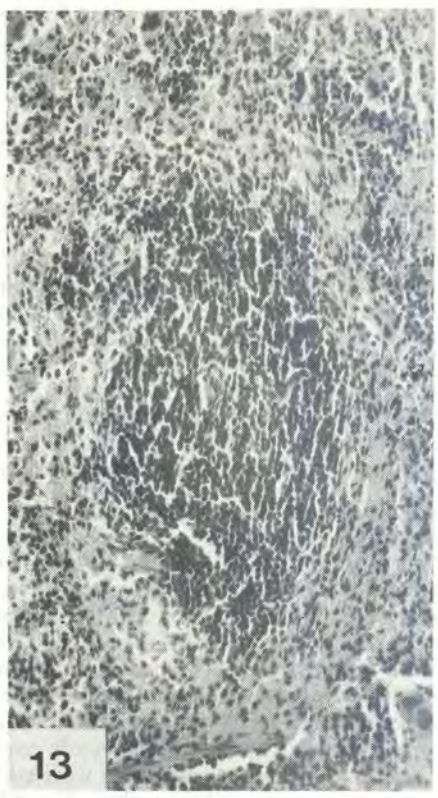
The sex steroids affected the thymus in different ways, the effect of estradiol being the most marked and even more striking than that of the glucocorticoids. This lymphocyte-depleting action of estradiol has been reported using puppies and rats as experimental animals (Dougherty, 1952). The same publication reported that testosterone propionate had a similar effect on the thymus to that of estradiol, but our work does not confirm this. A dosage effect may be operative here for, although the mass of the 2 steroids given was of the same order of magnitude, estradiol is known to be the more potent steroid.

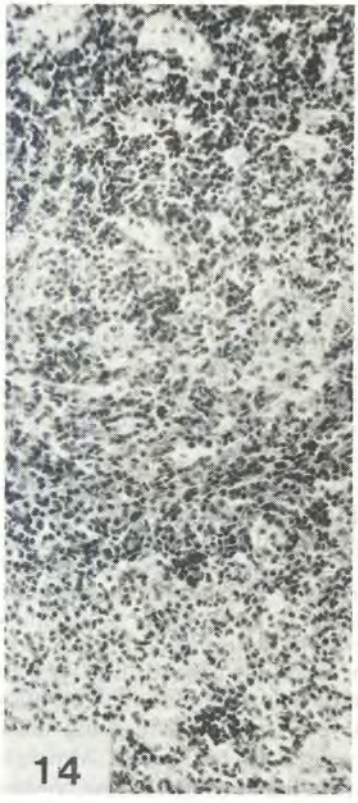
The spleen

1. Mice injected with SRBC (alone or with saline): At 24 h large Malpighian bodies were already present with many active germinal centres (Fig. 11) containing many mitotic figures. By Day 5 the Malpighian bodies were relatively large with condensed peripheries of small lymphocytes and with many germinal centres showing active mitosis and phagocytosis, indicating a well established lymphocyte turnover.
2. Mice injected with SRBC plus oil suspending medium: No visible changes occurred in the spleen.
3. Mice injected with SRBC plus 1 or 4 mg aqueous or oil suspensions of cortisol: There was no difference between the oil and aqueous suspensions. The regimen of 1 mg corticosteroid caused much more phagocytosis in the germinal centres and pyknosis of nuclei at 24 h (Fig. 12) than was observed in the SRBC-injected controls; and the cords of Billroth, especially towards the trabeculae, contained numerous darkly-staining lymphocytes which appeared very distinct. The 4 mg dosages gave a greater effect at 24 h. The Malpighian bodies were very small and compact, and contained very pyknotic nuclei. The cords of Billroth were affected as before. Germinal centres were absent and little phagocytosis was noted (Fig. 13). The marginal zones were hyperaemic, with an extended reticular cell network. These zones appeared to almost encapsulate the Malpighian bodies. There was an increase of neutrophils in the sinusoids. On Day 5 hardly any germinal centres could be seen in the spleen.









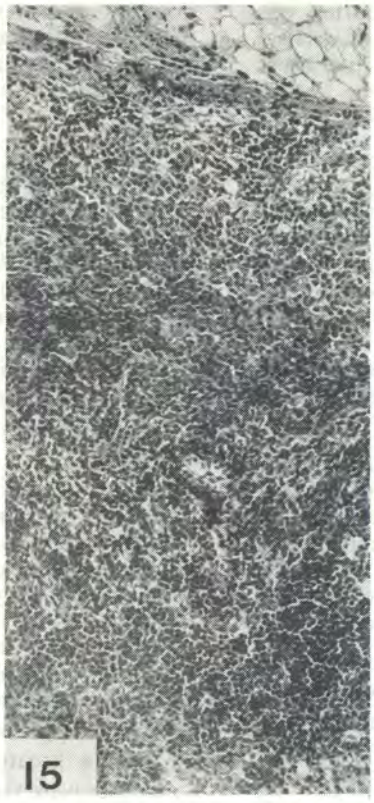


FIG. 11 Spleen: 24 h after SRBC (alone or plus saline) revealing large Malpighian bodies with germinal centres. ×200
FIG. 12 Spleen: 24 h after SRBC plus 1 mg doses of cortisol revealing some pyknosis of nuclei and some phagocytosis in germinal centres. ×200
FIG. 13 Spleen: 24 h after SRBC plus 4 mg doses of cortisol revealing pyknosis of nuclei with very ill-defined germinal centres and some phagocytosis. The latter was difficult to see. The marginal zone is hyperaemic and well demarcated. ×200
FIG. 14 Thymus: Day 5 after SRBC plus 2 mg doses of Efcortolan with cortex denuded of lymphocytes and macrophages vacuolated and filled with yellowish granules. The latter are not visible at this magnification. The medulla contains only a few lymphocytes. ×200
FIG. 15 Thymus: Day 5 after SRBC plus 1 mg doses of estradiol revealing a hardly recognizable cortex and medulla with marked pyknosis throughout. ×150
FIG. 16 Thymus: Group A, 4 h after 4 mg dose of cortisol, showing pyknotic and karyorrhectic nuclei clustered around reticular cells of cortex with phagocytosis mainly in the cortex. ×500

4. Mice injected with SRBC plus Betsolan: Essentially the same results were obtained at the 1,0 mg dose as with cortisol.
5. Mice injected with SRBC plus Efcortolan: On Day 5 small germinal centres were present here and there in the spleen, but Malpighian bodies consisted mainly of aggregations of small lymphocytes concentrated round the central arteries.
6. Mice injected with SRBC plus progesterone: At Day 5 only occasional germinal centres were present, and these were not very obvious. However, the lymphocytes in the Malpighian bodies, with their vesicular nuclei, were prominent and could possibly have been increased in number.
7. Mice injected with SRBC plus estradiol or testosterone propionate: In both cases the spleens had only small Malpighian bodies with ill-defined germinal centres on Day 5.

One may conclude therefore that all corticosteroid preparations tested affected the spleen by appearing to depress the normal response of the latter to antigen. This response was evinced by the spleens of mice treated with SRBC alone, and was maximal at Day 5, the time of maximum splenic enlargement after injection of this antigen (Hellig & Waldek, 1974). The effect of cortisol in depressing the formation of germinal centres after injection of SRBC is in agreement with the work of Bjørnboe *et al.* (1951) who showed a similar effect with the use of polyvalent pneumococcal vaccine as antigen in rabbits.

The effect of the other steroids was not so clear-cut, but both estradiol and testosterone propionate appeared also to depress the splenic immune response.

Time study on the effect of cortisol

Since cortisol brings about maximum depression of PFCs when the first injection is given 6–8 h before SRBC (Hellig & Waldek, 1974), it was considered important to investigate early changes in lymphopoietic tissues more thoroughly. This series of experiments was also designed to differentiate clearly between the changes brought about by cortisol alone as compared to its effects when superimposed on the immune response to SRBC. Group A received cortisol alone, Group B cortisol plus SRBC, and Group C SRBC alone.

The liver

Groups A and B: The glycogen vacuoles previously noted were present after steroid injection.

Group C: The only notable feature was some vacuolization different to the glycogen vacuolization, and probably related to nutrition.

All 3 groups showed varying degrees of polyploidy, a normal feature in the young mouse.

The thymus

Group A: By 4 h after the 1st steroid injection the thymus already showed the presence of pyknotic and karyorrhectic nuclei which were clustered round the reticular cells of the cortex and medulla, and excessive phagocytosis of nuclear debris by phagocytes mainly of the cortex (Fig. 16). By 8 h the destruction of lumphocytes in both cortex and medulla became very extensive. At 24 h phagocytosis could only be seen in the cortex and the depletion of cortical lymphocytes had become very marked. The cortex was very narrow and could be distinguished by almost complete absence of lymphocytes (cf. Fig. 9 of 1st experiment). By 48 h the cortex was virtually completely depleted of lymphocytes and endodermal reticular cells, and vacuolated macrophages were distinctly visible. Nuclear debris was only occasionally observed in the macrophages. This maximum degree of depletion was present for the next 2 days. During this time and the succeeding 3 days the corpuscles of Hassal were observed to have large lumina (Fig. 17). The period of thymus depletion lasted 2–3 days. Twenty-four h later a slight increase in the number of lymphocytes in the medulla and cortex (Fig. 18) indicated the beginning of a regenerative phase. By Day 7, i.e. 5 days after the last steroid injection, the thymus had a normal appearance. Both cortex and medulla were packed with lymphocytes and the corpuscles of Hassal were small and indistinct.

Group B: The cortex became completely depleted of lymphocytes, as it did in Group A. The only difference was that the medullary lymphocytes appeared to be spared to a greater degree during the period of maximal depletion of cortical lymphocytes. Fig. 19 illustrates the very marked difference between cortex and medulla at 48 h.

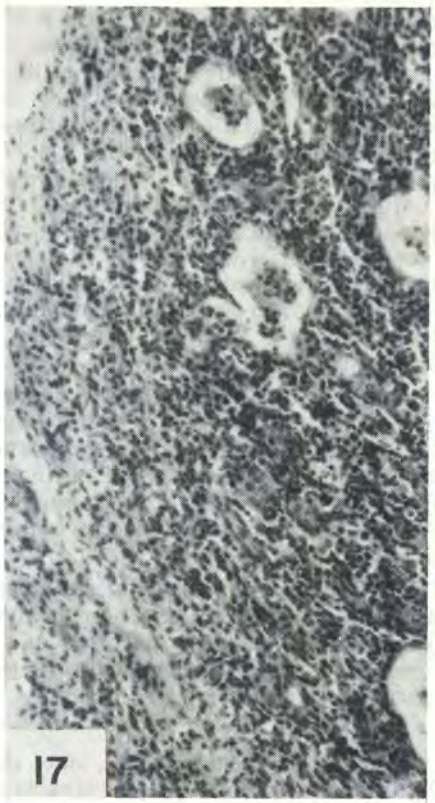
Group C: The thymus invariably presented a normal and active appearance, i.e. no depletion of lymphocytes, pyknosis or phagocytosis occurred at any stage. The cortex and medulla remained very well defined throughout the period of study.

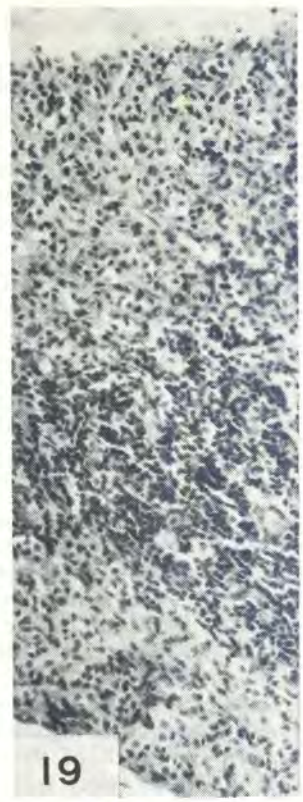
The spleen

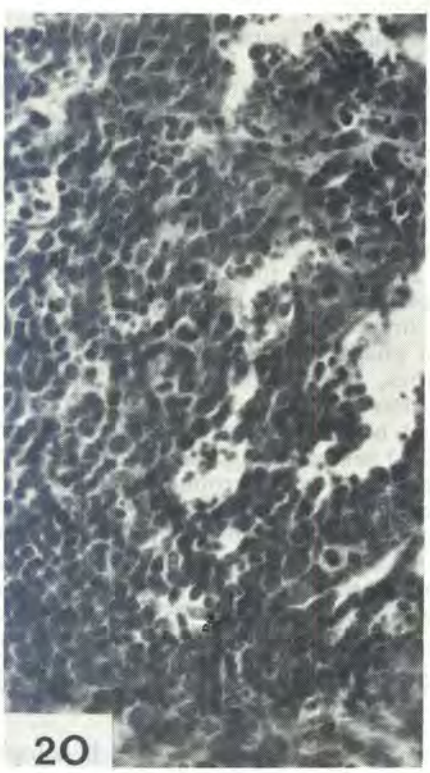
Groups A and C will be discussed first, since they represented 2 extremes with Group B occupying an intermediate position.

Group A: Within 4 h excessive phagocytosis and destruction of lymphocytes were observed in the Malpighian bodies (Fig. 20). No comparable change was observed in the red pulp. The high level of phagocytic activity was still present at 8 h, with all lymphocyte nuclei becoming pyknotic. By 12 h the phagocytic activity had abated. The red pulp revealed patches of darkly staining lymphocytes (cords of Billroth?) with lighter areas in between (sinusoids?) (Fig. 21). By 24 h only 2 to 3 germinal centres were seen throughout the lobule. However, both the 2nd and 3rd injections of cortisol were followed by a resumption of phagocytosis of nuclear debris in the germinal centres, which again died down after a further 24 h. After 56 h the number of lymphocytes in the Malpighian bodies was tremendously increased, the few germinal centres being filled with small lymphocytes. The number of neutrophils in the red pulp was also increased. By 72 h the spleen, on the whole, presented a fairly normal appearance, with a few active germinal centres. The neutrophils remained fairly numerous in the red pulp. From this time on—the period of recovery of mass (Hellig & Waldek, 1974)—the spleen showed an essentially normal appearance, with the white pulp increasing slightly, while some Malpighian bodies developed germinal centres.

Group C: The 1st specimens for histological examination were taken 4 h after the SRBC injection. Many small areas of intensely dark staining lymphocytes were seen scattered around the trabeculae. The lymphocytes in the Malpighian bodies were larger and less intensely stained, a feature which was particularly noticeable in the germinal centres that were present. Phagocytosis was also seen in the germinal centres, but at the same time lymphoblasts showed many mitotic figures and appeared to be reacting to the SRBC stimulus. At 40 h after the







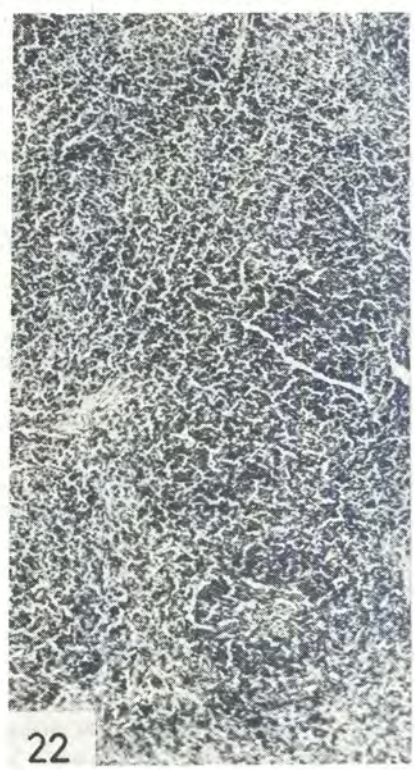


FIG. 17 Thymus: Group A, 48 h after 4 mg doses of cortisol. The cortex reveals some phagocytosis and partial depletion of lymphocytes. The corpuscles of Hassal show enlarged lumina. ×200

FIG. 18 Thymus: Group A, 72 h after 4 mg doses of cortisol, illustrating a slight increase of lymphocyte numbers in medulla and cortex. ×200

FIG. 19 Thymus: Group B, 48 h after 4 mg doses of cortisol and 32 h after SRBC, showing marked discrepancy between cortex and medulla. ×200

FIG. 20 Spleen; Group A, 4 h after a 4 mg dose of cortisol, with excessive destruction and phagocytosis of lymphocytes in Malpighian bodies. ×500

FIG. 21 Spleen: Group A, 12 h after a 4 mg dose of cortisol, showing aggregation of lymphocytes in red pulp and absence of germinal centres in white pulp. ×75

FIG. 22 Spleen: Group C, 72 h after SRBC, revealing active germinal centres and general increase in lymphoid tissue. ×75

SRBC injection the Malpighian bodies were large and well developed (cf. Fig. 11 of 1st experiment). Although fairly small, the germinal centres showed very active mitosis. In the subsequent time intervals the Malpighian bodies with numerous active germinal centres developed further. By 72 h they dominated the picture (Fig. 22) and at 96 h there was an obvious increase in lymphoid tissue. This is in accord with the fact that at this time the immune response is at its peak and the spleen mass has increased (Hellig & Waldek, 1974). The germinal centres remained active, phagocytosis and mitosis being prominent, till the end of the time period studied, i.e. Day 7.

Group B: At 4 h after SRBC (12 h after the first injection of corticosteroid) karyorrhexis and phagocytosis of nuclear debris were very marked in the white pulp; cf. Group A, where phagocytosis had ceased, and Group C, where germinal centres were already becoming apparent at this time. By 24 h after the first corticosteroid injection a general pyknosis of nuclei was apparent, but a few small germinal centres were visible, and these contained mitotic figures. The germinal centres remained evident despite further corticosteroid injections, but contained small lymphocytes and relatively few mitotic figures compared to Group C, and there were many pyknotic nuclei in the Malpighian bodies. However, by 80 h, as in Group A, the spleen showed that the effect of steroid was diminishing. Thereafter the white pulp increased in quantity and the germinal centres became well developed. The lymphocytes did not appear to be quite as abundant as in Group C. The number of neutrophils in the red pulp increased, as they did in Group A.

It should be noted that with corticosteroid treatment 6–8 h before SRBC injection, very active phagocytosis of lymphocytes is taking place in the spleen at the time that the antigen is introduced. The ontogeny of the splenic immune response may be interfered with in 2 probable ways. Firstly through the destruction of B cell precursors of PFCs, and secondly through possible deficient antigen uptake by macrophages already engaged in active phagocytosis.

Lymph nodes

A complete set of lymph nodes was not collected. Moreover histological sections were mostly very unsatisfactory because of the minute size of the tissue.

Group A: The 1st lymph node in this group was obtained at 12 h, and the only change noted was excessive phagocytosis of nuclear debris in germinal centres and a slight degree of pyknosis of lymphocyte nuclei. At 24 h some phagocytosis could still be detected and no distinct germinal centres were visible. By 32 h the reticulo-endothelial cells in the sinuses presented signs of increased activity, namely enlargement and vacuolation. The vacuoles contained a brownish substance, probably lipofuscin, and in the barely defined germinal centres some phagocytosis of nuclear debris was still taking place. By 48 h the node had become extremely small and it was difficult to extract any information from the section. At 80 h no sharp distinction could be made between cortex and medulla. However, by 96 h the lymph node appeared normal and had a well developed cortex and medulla. In the succeeding sections this appearance was maintained, with the development of fairly numerous germinal centres.

Group C: The 1st section (16 h after SRBC) showed no germinal centres as yet, but only dense lymphocytes. By 80 h the cortex appeared wide and well-packed with lymphocytes. Active germinal centres were present. These increased in number as time progressed, and phagocytosis was a prominent feature within them.

Bone marrow

Group A: On the 1st day the bone marrow of this group revealed phagocytosis of nuclear debris and the beginning of a progressive relative increase of neutrophilic promyelocytes. Their numbers reached a maximum on Day 4, whereafter they gradually declined to normal. Although a drop in lymphocytes was anticipated (Morrison & Toepfer, 1967; Bennet & Cudkowicz, 1968) this could not be confirmed because it proved difficult to distinguish them from polychromatophil erythroblasts. Initially no depletion of erythrocyte precursors was noted, but normoblasts soon started decreasing, reaching a minimum on Day 2 and 3. Thereafter they increased in numbers and slightly exceeded normal levels by Day 6. Plasma cells were not increased. The basophilia of erythrocyte progenitors was also more intense.

Group B: This group showed very much the same picture as Group A. Thus neutrophil myelocytes were also increased on Days 2–7 and a very marked basophilia of erythrocyte progenitors was observed. Normoblasts again decreased during the 2nd to 5th days. Plasma cells rose in number towards the end of the series.

Group C: This group revealed very much less change than the preceding 2 groups. The proportion of myelocytes was indeed slightly increased, but not nearly as much as after cortisol treatment. Plasma cells also appeared to increase in numbers.

The phenomenon of neutrophilic promyelocyte increase in corticoid treated animals has been noted previously (Morrison & Toepfer, 1967), but a satisfactory explanation is still lacking. The transient decrease in the number of normoblasts after steroid treatment has not been reported previously, but the subsequent increase may be explained as an erythrocytosis resulting from the eosinopenia produced by cortisol. White & Dougherty (1945) had postulated that such an erythrocytosis might be expected after corticosteroid treatment. The rise in plasma cells in the 2 SRBC treated groups may be ascribed to the fact that these cells are antibody producers and should be expected to rise during the immune response.

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