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The Morphology of Immune Reactions in Normal, Thymectomized and Reconstituted Mice

II. THE RESPONSE TO OXAZOLONE

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Summary. The response to oxazolone, painted on the skin, has been studied in the regional lymph nodes of normal adult CBA/H mice and of thymectomized, X-irradiated, bone-marrow injected mice with or without a reconstituting thymus graft. The thymus grafts carry a marker chromosome so that, in a reconstituted animal, cells derived from bone marrow and from the thymus can be distinguished. The response to oxazolone appears to be biphasic and both thymus-derived and bone-marrow-derived cells participate in it. In the first stage, normal mice show a massive proliferation of thymus-derived cells in the paracortex which reaches a peak on day 4. This reaction is absent in thymectomized mice but is restored if animals are grafted with a syngeneic thymus. The second stage consists of proliferation of bone-marrow derived cells which is maximal on day 8 and is then sustained, at a slightly lower level, for the rest of the experiment. It coincides with germinal centre formation and hyperplasia of the medullary cords—changes which are best seen in normal and reconstituted mice though they are also present to a lesser extent in thymectomized animals. The part played by bone-marrow derived elements in the immune response to oxazolone is not known but that there is a considerable proliferation of these cells in the later stages in the present experimental model is certain.

INTRODUCTION

In an extension of an earlier investigation (Turk and Stone, 1963), Oort and Turk(1965) gave a detailed account of the morphological response in the lymph nodes of guinea-pigs painted with the skin-sensitizing agent 2-phenyl-4-ethoxymethylene-5-oxazolone (oxazolone). The main feature was an intense hyperplasia which was virtually confined to blast cells in the paracortex. Medullary plasmocytosis was noted towards the end of the period of observation and 'very many plasma cells were seen' 6 days after sensitization. Other structures were apparently not involved and the follicles and germinal centres were not, at that time, 'affected to any important extent by the sensitization process'. Oort and Turk recorded autoradiographic findings with tritiated thymidine and descriptions of the ultrastructure (De Petris, Karlsbad, Pernis and Turk, 1966) and cytochemistry (Diengdoh and Turk, 1966) of the paracortical cells in guinea-pigs.

Shortly after the initial work by Turk, various investigators demonstrated that the integrity of the paracortical region of lymph nodes was dependent upon the presence of an intact and functioning thymus. Selective depletion of the paracortex was produced in neonatal mice by thymectomy but an intravenous injection of syngeneic thymic cells repaired this deficiency, the thymocytes localizing preferentially in the depleted region (Parrott, de Sousa and East, 1966). A similar repopulation was observed in thymectomized mice which received a thymus graft labelled with tritiated thymidine (Parrott and de Sousa, 1967). These observations suggested that the response to oxazolone, concentrated almost entirely in the paracortex, might be suppressed by prior thymectomy. This was demonstrated by Parrott and de Sousa (1966; see also Parrott, 1967) who showed that the paracortical reaction to oxazolone did not take place in thymectomized mice although such animals developed hyperplastic changes in the follicles and medullary cords when immunized with pneumococcal polysaccharide.

In order to investigate the response to oxazolone in more detail, it was decided to compare its effect on normal, thymectomized and reconstituted mice as in previous experiments with heterologous erythrocytes (Davies, Carter, Leuchars, Wallis and Koller, 1969). In this way the involvement of thymus-derived cells in the reaction can be studied more directly by means of a genetic rather than an isotopic marker of thymic lymphocytes; some of the disadvantages of the latter labelling technique have already been pointed out by Parrott and de Sousa (1967). Furthermore, by using reconstituted mice with appropriate cytological markers, it can be determined whether two separate populations of cells, of thymus and of bone-marrow (graft) origin, participate in the response—a suggestion which has been considered elsewhere (Davies *et al.*, 1969).

MATERIALS AND METHODS

The experimental design was the same as that adopted in previous experiments where the immune response to sheep erythrocytes was analysed (Davies, *et al.*, 1969). CBA/H male mice were thymectomized at 8 weeks of age. Two weeks later, they were subjected to 850 r total body irradiation from a 220 kV X-ray machine. Within 3 hours, an intravenous injection of 5×10^6 cells of syngeneic (CBA/H) bone-marrow was given. These mice were then grafted with a single lobe of a CBA/H-*T6T6* neonatal thymus which was implanted under the capsule of the left kidney. Approximately 120 animals were prepared in this way and they will be referred to as *reconstituted* mice. A further sixty mice, which were not given thymus grafts, will be called *deprived*. The method of preparation of these chimaeras is described in detail by Davies, Leuchars, Wallis and Koller (1966). A group of sixty *normal* male mice of the CBA/H strain was also studied.

Cytological and histopathological studies were carried out as follows:

Cytology

Reconstituted mice, three in a box, were left until 50 days after irradiation and implantation of thymus grafts. Each mouse was then painted with oxazolone, approximately 0·5 ml of a 10 per cent solution in absolute alcohol being divided equally between four sites—the two fore feet and two sites high up on the back. Each day for 10 days following this treatment, a box of mice was taken and the internal and external axillary lymph nodes were removed for cytological analysis. Twenty-eight axillary lymph nodes from unpainted control mice were also taken over the same time course. (It was not practicable

to do within-box controls as oxazolone is easily transferred from one animal to another.) Each lymph node was dealt with separately so that about twelve experimental and two to four control nodes were examined each day. The preparation of cells for cytological analysis was based on that described by Ford (1966). The slides from each node were coded and inspected independently by different workers. Distinction was made, as before, between CBA/H-*T6T6* cells derived from the thymus graft and CBA/H cells derived from the bone-marrow (Davies, Leuchars, Wallis, Marchant and Elliott, 1967). Where possible, fifty dividing cells were scored on each slide.

Histopathology

Reconstituted, deprived and normal mice were placed five in a box. Fifty days after irradiation, five mice in each group were weighed and killed. Axillary lymph nodes and thymus tissue (if present) were removed, weighed, and fixed in Bouin's solution. Paraffin sections were prepared at 5 μ and stained with haematoxylin and eosin and, in some instances, with Giemsa and Gordon and Sweets' silver impregnation technique for reticulin fibres. All sections were coded and examined independently. All but fifteen of the remaining mice were painted with oxazolone as above. On each of the 10 subsequent days, five mice in each group were killed and dissected. On the 11th day, the fifteen remaining unpainted mice were killed and their axillary lymph nodes were fixed for histology; control mice were thus examined at both the beginning and end of the experiment.

Reconstitution with thymocytes

In mice reconstituted with a thymus graft, thymus-derived cells may be of two kinds; those known to emanate from the graft itself and those, of bone-marrow graft origin, which enter the thymus graft and subsequently emerge to behave in the same manner as fully accredited cells of thymic origin. Such a phenomenon can be investigated by a comparison between mice which have been reconstituted with thymocytes and similar animals which have received a thymus graft. Proper interpretation of the results of the experiments concerning the effect of oxazolone on thymus-graft reconstituted mice requires consideration of this point and, therefore, an appropriate experiment is included here. CBA/H mice were thymectomized, irradiated and injected with bone-marrow, as before, and then given either a thymus-graft or an intravenous injection of 30×10^6 thymocytes. The thymocytes were collected from 4-week-old mice of the CBA/H-*T6T6* strain while the thymus grafts were from 1- to 3-day-old mice of that strain (as before). Fifty days after their reconstitution, all the mice were painted with oxazolone and cytological analysis was carried out on the draining lymph nodes on days 2, 3, 4, 5 and 6.

RESULTS

The body weights, thymus–body weight ratios, and lymph node–body weight ratios on each day of the experiment are recorded in Table 1. The mean *body weights* showed a good deal of variation from day to day, particularly among the normal mice, but certain general trends are apparent. In all groups, there was an initial drop in weight after oxazolone was applied to the skin. The body weight of normal and reconstituted mice subsequently rose but the mean weight of the deprived mice remained low until day 7. By the end of the experiment, normal and deprived mice had regained or exceeded their original body weight but the weights of the reconstituted mice were below the pre-treatment values.

TABLE 1

THE MEAN BODY WEIGHT, THYMUS–BODY WEIGHT RATIOS AND DRAINING LYMPH NODE–BODY WEIGHT RATIOS OF NORMAL, DEPRIVED AND RECONSTITUTED MICE (SEE TEXT) DURING A 10-DAY PERIOD AFTER PAINTING WITH OXAZOLONE (NOTE THAT DEPRIVED MICE HAVE NO THYMIC TISSUE)

	Starting control	Time (days) after painting									Finishing control
		1	2	3	4	5	6	7	8	9	10	
Body weight (g) $n \geqslant 3$												
1. Normal	26·7 ± 1·1	23·0 ± 0·0	28·3 ± 1·9	28·0 ± 2·0	28·7 ± 0·9	31·0 ± 2·6	30·0 ± 3·6	29·3 ± 1·3	27·3 ± 3·1	28·3 ± 1·9	33·5 ± 1·9	30·8 ± 3·8
2. Deprived	29·3 ± 1·3	26·7 ± 2·8	28·0 ± 1·7	28·0 ± 2·6	26·8 ± 2·5	26·5 ± 2·9	27·7 ± 2·0	26·5 ± 2·6	28·5 ± 0·7	28·0 ± 1·0	29·3 ± 1·3	29·0 ± 2·2
3. Reconstituted	28·3 ± 2·4	26·0 ± 1·4	25·3 ± 3·7	28·0 ± 0·0	28·0 ± 1·7	28·0 ± 2·6	28·7 ± 0·9	27·3 ± 1·9	29·7 ± 0·9	28·3 ± 3·3	26·7 ± 1·0	29·2 ± 1·7
Thymus weight/body weight $\times 10^4$ $n \geqslant 3$												
1. Normal		9·71 ± 2·75	10·83 ± 4·92	6·40 ± 0·54	6·29 ± 1·17	6·05 ± 0·58	6·35 ± 0·73	8·14 ± 0·50	6·80 ± 1·61	6·67 ± 0·74	8·09 ± 1·02	9·74 ± 2·77
2. Reconstituted	3·98 ± 0·57	3·67 ± 0·00	3·54 ± 0·62	2·23 ± 0·65	2·70 ± 1·24	2·82 ± 0·47	2·89 ± 0·16	3·57 ± 0·45	3·37 ± 0·19	3·42 ± 1·28	3·37 ± 0·67	3·33 ± 0·77
External axillary lymph node weight/ body weight $\times 10^4$ $n \geqslant 6$												
1. Normal	0·82 ± 0·26	1·98 ± 0·21	2·67 ± 0·72	4·73 ± 0·78	4·92 ± 0·88	5·31 ± 0·61	4·39 ± 0·62	4·44 ± 1·07	4·20 ± 0·82	4·34 ± 0·95	4·29 ± 0·65	1·00 ± 0·26
2. Deprived	0·69 ± 0·21	0·97 ± 0·28	1·48 ± 0·06	1·86 ± 0·24	1·90 ± 0·42	2·00 ± 0·41	1·58 ± 0·25	1·55 ± 0·38	1·50 ± 0·32	1·89 ± 0·91	1·21 ± 0·26	0·56 ± 0·22
3. Reconstituted	1·03 ± 0·30	1·75 ± 0·06	3·18 ± 0·46	3·59 ± 0·47	4·16 ± 0·98	4·47 ± 0·74	4·46 ± 0·39	4·18 ± 0·19	3·83 ± 0·67	3·37 ± 0·67	3·01 ± 0·43	1·26 ± 0·39

The starting and finishing controls were not painted. Standard deviations are given for each mean.

The *thymus–body weight ratios* showed a fall in normal and reconstituted mice at day 3, more marked in normal mice (which had approximately twice as much thymus tissue as the reconstituted animals). The *lymph node–body weight ratios* (Fig. 1) were strikingly raised in normal mice, with a more than two-fold increase apparent after 1 day. The peak was reached at day 5 and the increase was sustained, at a lower level, for the rest of the experiment. A similar but less marked response was seen in reconstituted mice. Deprived mice, by contrast, showed only a slight increase in lymph node–body weight ratios although it is interesting that two features observed in the other experimental groups were preserved: maximum values still occurred on day 5, and the increase was sustained (albeit at a very low level until) the end of the experiment.

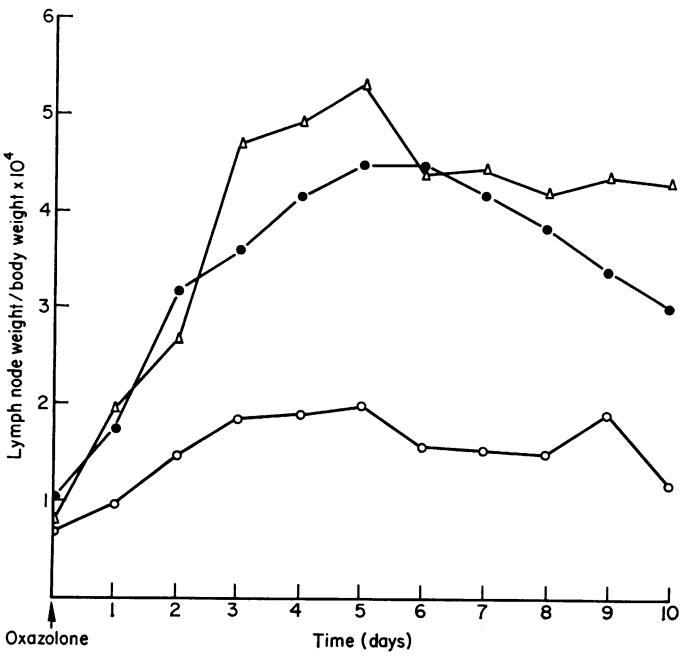

FIG. 1. The draining lymph node–body weight ratios of normal (△), deprived (○) and reconstituted (●) mice (see text) during a 10-day period after painting with oxazolone.

HISTOPATHOLOGY

Lymph nodes

Normal mice. One hundred and thirty-two axillary lymph nodes were examined from thirty-five normal mice: 113 nodes from twenty-nine test mice, painted with oxazolone, and nineteen nodes from six untreated control animals.

Axillary lymph nodes from control mice were of normal size and cellularity. The follicles, paracortical regions and medullary cords were unremarkable in appearance.

The changes evoked by oxazolone were strikingly consistent and, at the peak of the response, occurred in virtually all nodes which were examined. On day 1, the nodes were enlarged and contained increased numbers of big basophilic blast cells in the paracortex. These cells became more numerous and, on days 2 and 3, the paracortex had expanded, compressing the normal elements of the pulp into a narrow rim at the margin of the node.

The centres of such nodes were pale and composed of dense sheets of blast cells (Fig. 2); mitotic figures were abundant. Silver stains showed that the normal reticulin framework was frayed and partially disrupted by the expanding paracortex and was compressed

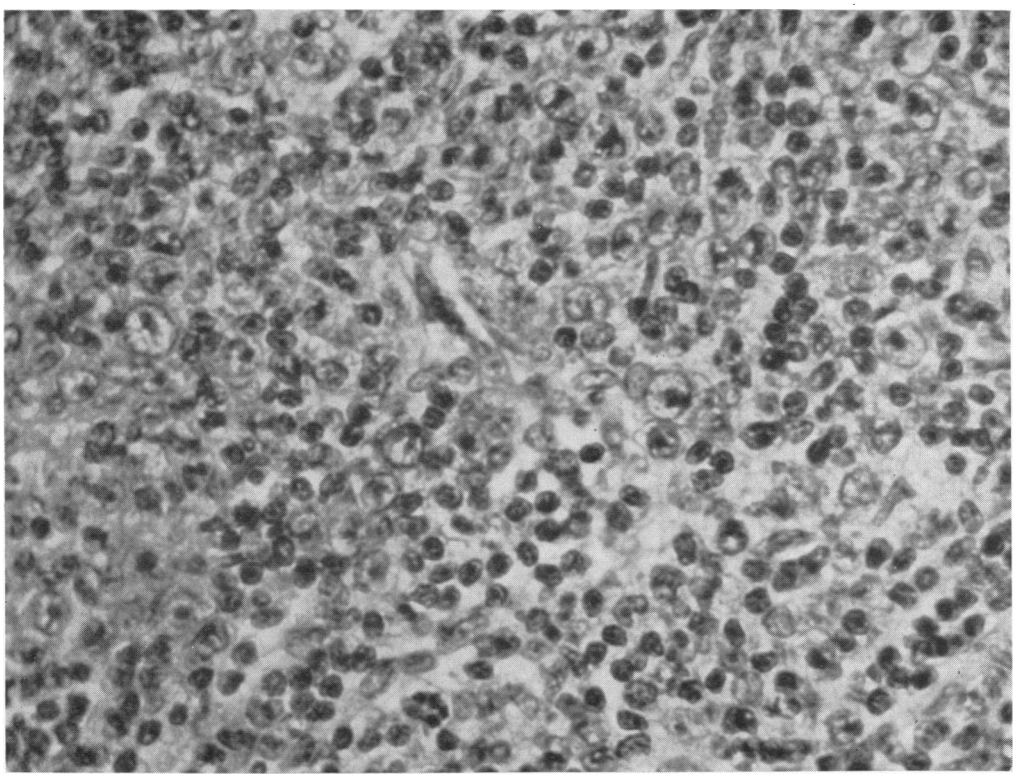

FIG. 2. Normal mouse, axillary lymph node, 3 days. Massive hyperplasia of paracortical blast cells. H & E, ×630.

peripherally (Fig. 3). Paracortical hyperplasia was usually maximal on day 4; thereafter, it began to decline. The demarcation between compressed normal pulp at the edge of the nodes and the central mass of reactive paracortical elements became less apparent. Normal sinus patterns re-emerged and small follicles could be made out in several nodes, some of them with small but active germinal centres. On subsequent days, paracortical proliferation became less evident but cells in mitosis were present until day 8. Meanwhile, activity in follicles and medullary cords became prominent (Fig. 4). The follicles increased in number and size, and developed large germinal centres, which were typically irregular in outline; any surrounding cuff of small lymphocytes was difficult to recognize. There were numerous mitoses among germinal centre cells and, in the later stages, pyknotic cells and cell-debris increased in amount. The medullary cords were enlarged and contained increased numbers of plasma cell elements. At the end of the experiment, the paracortex was normal but some hyperplasia persisted in the follicles and (to a lesser extent) in the medullary cords.

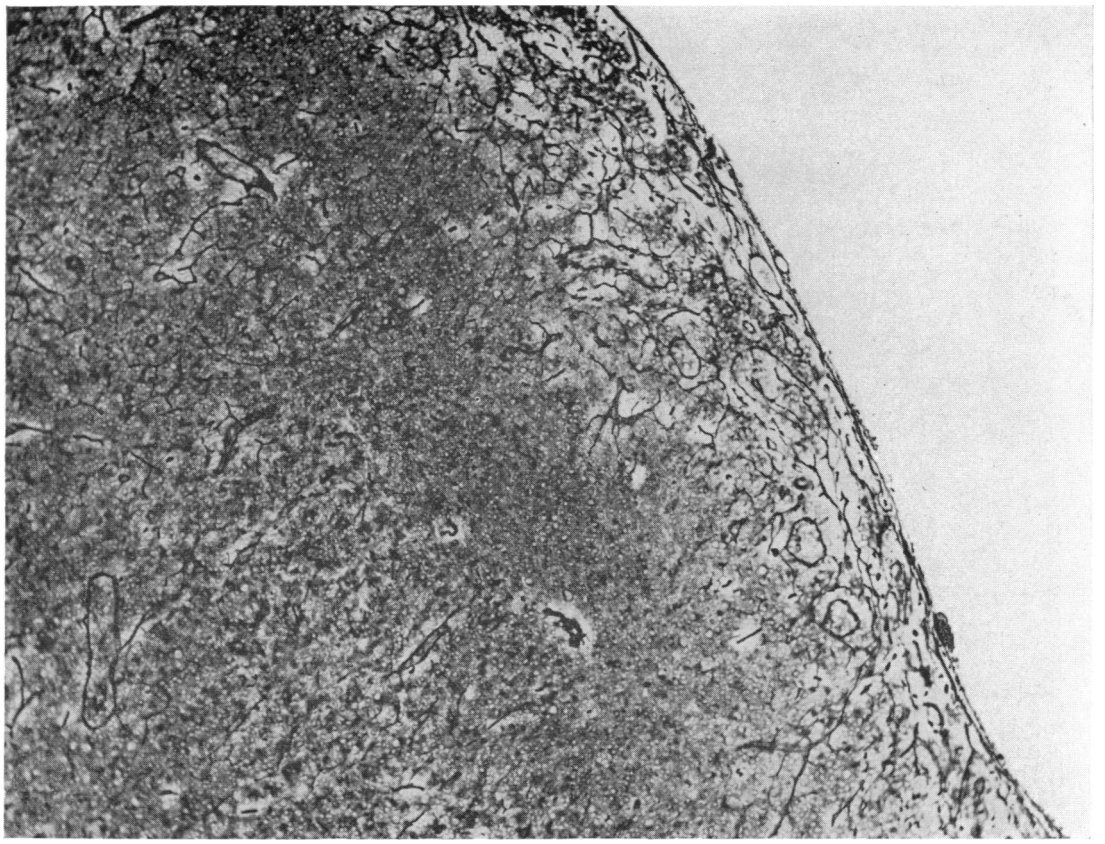

FIG. 3. Normal mouse, axillary lymph node, 3 days. Expansion and fragmentation of reticulin framework by hyperplastic paracortex. Silver impregnation (Gordon and Sweets'), × 160.

Deprived mice. Axillary lymph nodes were examined from thirty-five deprived mice: 112 nodes from twenty-nine treated mice and nineteen nodes from six untreated control animals.

Lymph nodes from control mice were small (see Table 1) with uniform hypoplasia of the paracortex and quiescent follicles and medullary cords.

Nodes from the test group of deprived animals were somewhat larger but showed negligible paracortical activity. A small number of blast cells was seen in the paracortex on the 2nd, 3rd, 4th and 5th days after the application of oxazolone but comparison of Fig. 5 with the previous Fig. 2, which show the paracortex in nodes from normal and deprived mice at 3 days, illustrates the feeble reaction in the deprived group. At about 4 days there was some enlargement of the follicles and medullary cords but this was short-lived and normal appearances were regained by the end of the experiment at 10 days.

Reconstituted mice. One hundred and twenty-two axillary lymph nodes were examined from thirty-seven reconstituted mice: 104 nodes from twenty-eight test mice and eighteen nodes from nine untreated control animals.

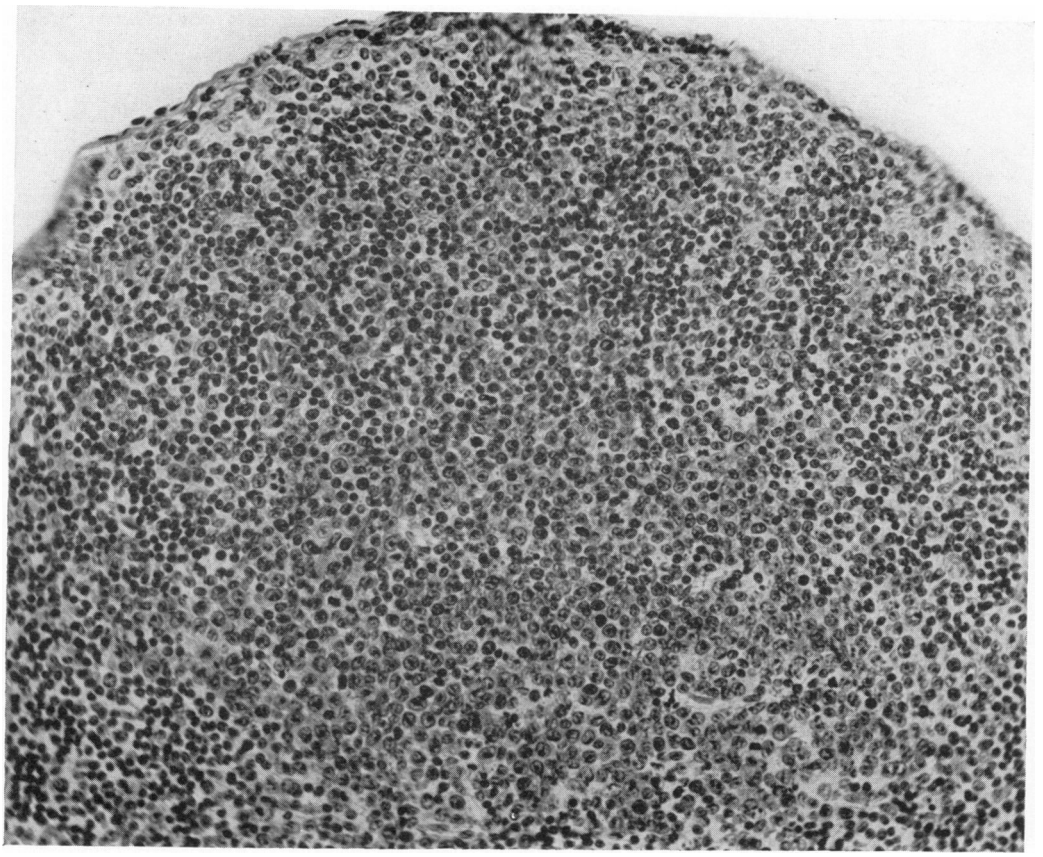

FIG. 4. Normal mouse, axillary lymph node, 5 days. Follicle with greatly enlarged germinal centre, poorly demarcated from the surrounding cortex. H & E, ×300.

Lymph nodes from untreated reconstituted mice were of normal size and cellularity. The follicles, paracortex and medullary cords were unremarkable.

Lymph nodes from treated reconstituted mice developed a sequence of reactive changes broadly similar to that previously described in normal mice painted with oxazolone. Initially, however, the response in the draining nodes developed a little more slowly than in normal mice. On day 1, for example, fewer blast cells were seen in the paracortex and the morphological response did not reach its peak until days 4 and 5 (Fig. 6). Appearances at this time were similar to those previously described: the nodes were enlarged and consisted of a central pale zone, composed of sheets of proliferating blast cells, and a narrow rim of compressed pulp at the periphery. Fragmentation and distortion of the reticulin framework was again noted. The paracortical proliferation declined on days 5 and 6 and, as in normal mice, follicles with active germinal centres became apparent at the edge of the nodes. Hyperplasia of follicles and, to a lesser extent, medullary cords persisted and signs of moderate activity were still present on day 10, at the end of the experiment.

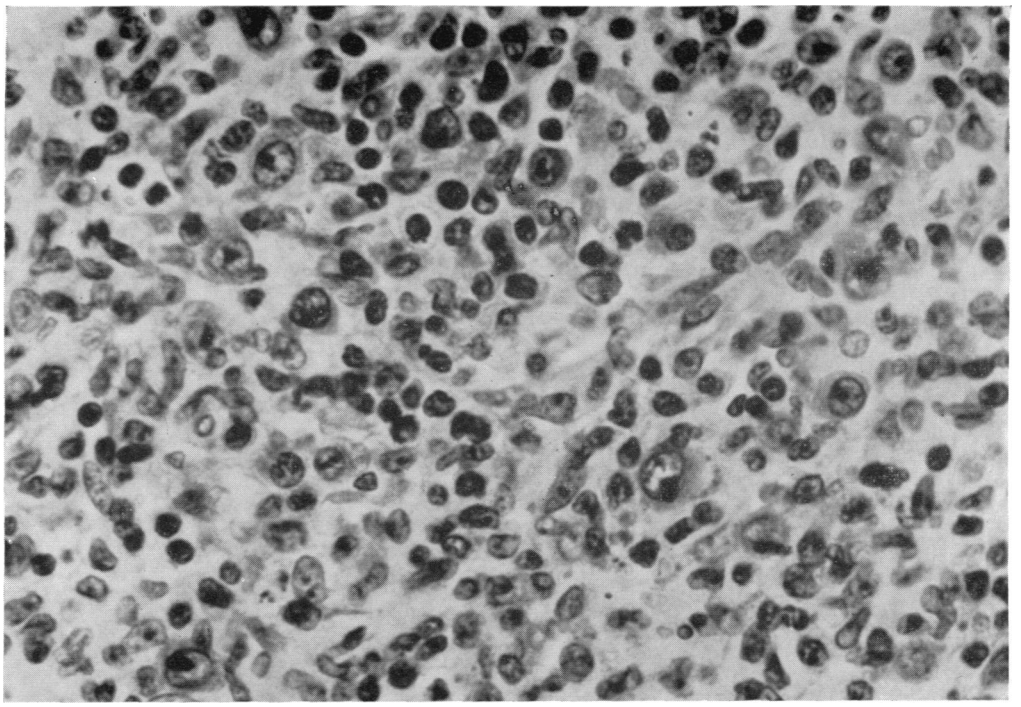

FIG. 5. Deprived mouse, axillary lymph node, 3 days. Slight proliferation of paracortical blast cells (compare with Fig. 2). H & E, ×630.

Skin

The sequence and intensity of the local changes induced in the skin by one application of oxazolone were similar in mice in the three experimental groups. Pronounced epidermal necrosis was observed as early as day 1, together with a dense intra-epidermal infiltrate of acute inflammatory cells which extended into the keratin layers. Pus cells were seen in the ostia of the hair follicles and around hair sheaths but there was little evidence of downward spread into the dermis (Fig. 7). Signs of extensive epidermal damage persisted for 3–4 days with superficial ulceration and micro-abscesses. The inflammatory infiltrate in the dermis became somewhat more prominent but the principal changes remained localized to the superficial parts of the skin. After 4 days there was rapid reconstitution of the epidermis and repair was almost complete by day 7. The epidermis was largely normal at this time, apart from a few patches of hyperplasia and hyperkeratosis; scanty infiltrates persisted in the dermis of a few animals but, by 10 days, dermal and epidermal structures were again normal.

CYTOLOGY

Thymus-graft reconstituted mice

The results of the cytological analyses are given in Table 2. Few mitoses were seen in axillary lymph nodes taken from untreated control mice and it is, therefore, reasonable to assume that cells scored in the nodes of test mice had been stimulated to divide as a result

TABLE 2

A RECORD OF THE DIVIDING CELLS SCORED IN THE DRAINING LYMPH NODES OF RECONSTITUTED MICE (SEE TEXT) DURING A 10-DAY PERIOD AFTER PAINTING WITH OXAZOLONE

Days after painting with oxazolone	No. of lymph nodes scored	Total No. of cells scored	No. of thymus-derived cells scored	Thymus-derived cells (per cent)
1	12	15	1	6·7
2	12	353	221	62·6
3	12	579	450	77·7
4	12	664	445	67·0
5	12	894	168	18·8
6	11	786	104	13·2
7	12	649	55	8·5
8	12	630	26	4·1
9	12	559	26	4·7
10	12	136	5	3·7
Unpainted controls	28	49	16	32·7

Cells were classified according to their origin from thymus or bone-marrow grafts.

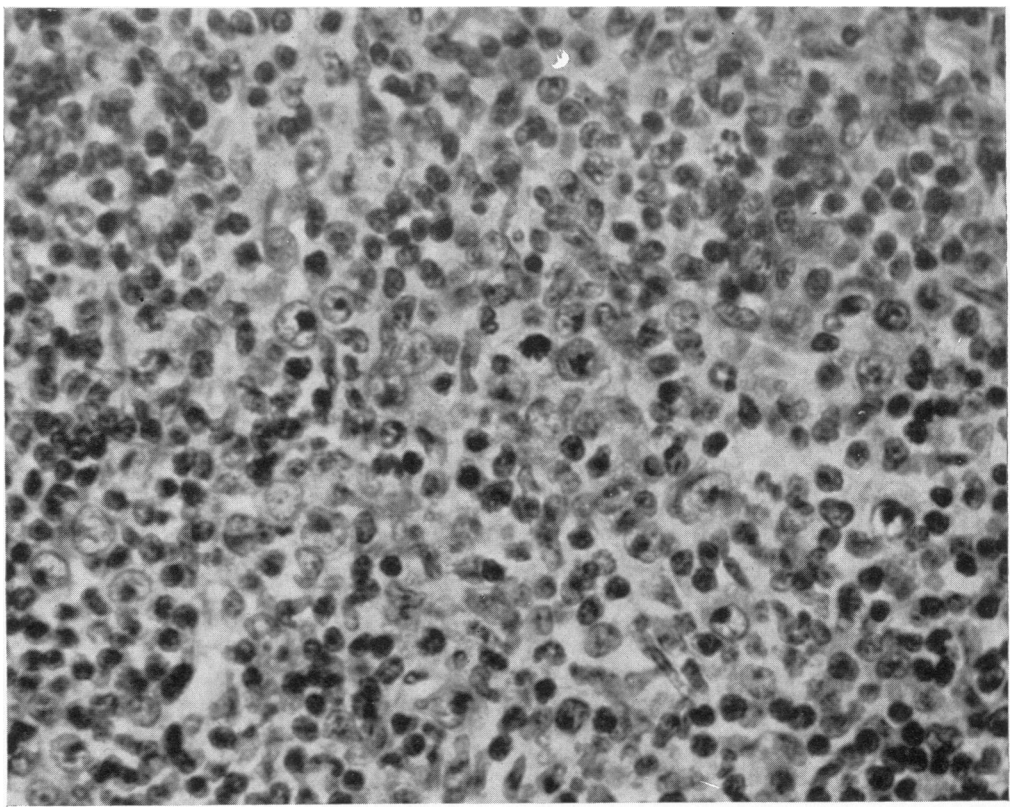

FIG. 6. Reconstituted mouse, axillary lymph node, 5 days. Extensive hyperplasia of paracortex. H & E, ×630.

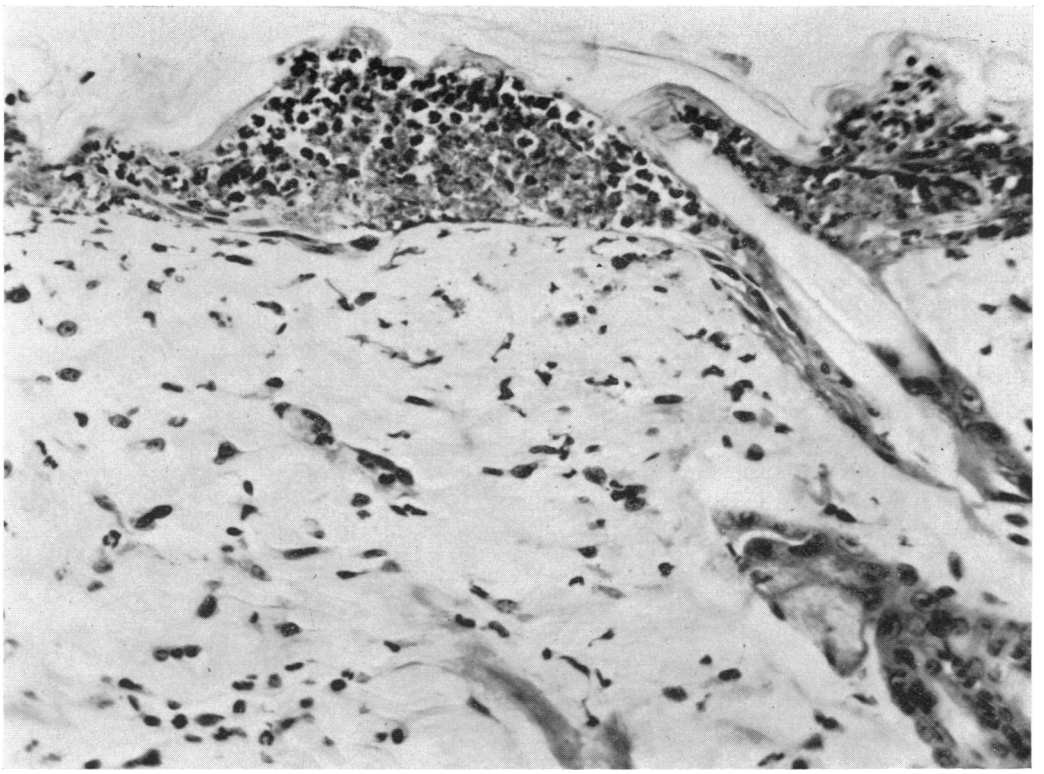

FIG. 7. Normal mouse, painted skin, 2 days. Extensive epidermal infiltration and necrosis. Acute inflammatory cells also extend round hair follicle but only scanty infiltrates are seen in the dermis. H & E, ×400.

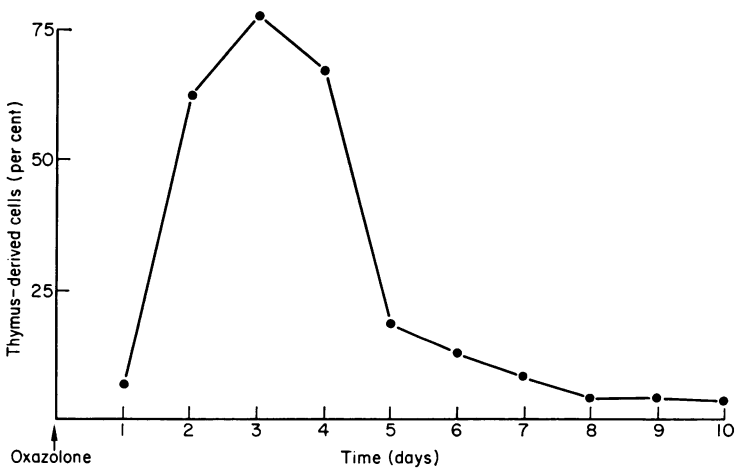

FIG. 8. The percentage of the dividing cell population known to derive from the thymus graft in the draining lymph nodes of reconstituted mice (see text) during a 10-day period after painting with oxazolone.

of oxazolone treatment. Fig. 8, showing the percentage of dividing cells of thymus-graft origin, demonstrates that mitoses of thymus-derived cells occurred predominantly on days 2, 3 and 4 after antigenic stimulation; subsequently, only a small percentage of cells scored was of this type. The expression of these results as proportions is potentially misleading as there were considerable changes in both lymph-node weight and mitotic activity during the course of the experiment. A more realistic appraisal of the situation derives from Fig. 9 on which are plotted the mean numbers of cells of each type scored. This means of quantitation, however, considerably underestimates the response (particularly at its peak) as may be deduced from Fig. 10. The underestimate arises because only two slides were made from each node, irrespective of its size, and on each slide only fifty dividing cells were scored. However, if it is accepted that the lymph node weight changes are related to the mitotic activity (the correspondence in shape between the two lines on Fig. 10 strongly suggests this) then the results given in Figs. 8–10 collectively provide a fair picture of the mitotic events in lymph nodes after contact with oxazolone. It can be said that considerable mitotic activity is induced which is predominantly of thymus-derived cells for the first few days, but thereafter comes to consist almost entirely of bone-marrow derived cells. Elevated numbers of thymus-derived cells in mitosis were, however, recorded for most of the 10-day observation period.

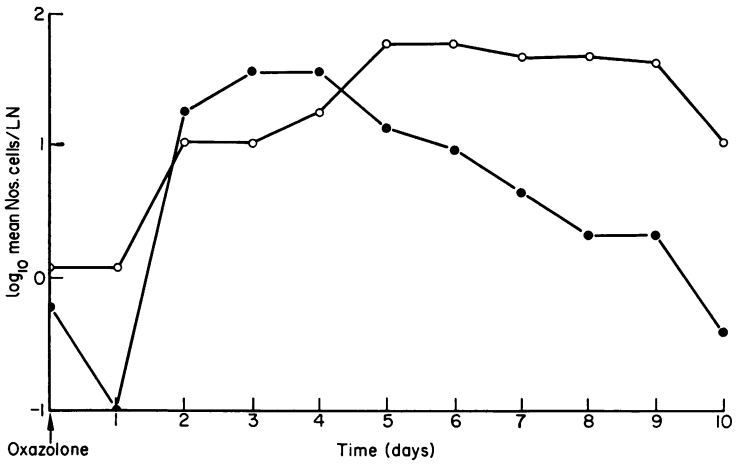

FIG. 9. The logarithms, to the base ten, of the mean numbers of dividing cells known to have derived from the thymus (●) and bone marrow (○) grafts in samples of the draining lymph nodes of reconstituted mice (see text) during a 10-day period after painting with oxazolone.

Thymocyte reconstituted mice

It can be seen in Fig. 11 that, at the peak of thymus-cell reactivity, 95 per cent of the cells in mitosis were derived from the injected thymocyte population whereas only 77 per cent of reacting cells were of known thymus origin in thymus-grafted mice. The other portion of Fig. 11 shows that the responses in the two different kinds of mice are quantitatively similar at the times of peak activity. A reasonable interpretation of this experimental result is that nearly all the cells in mitosis at 3 days were in fact thymus-processed cells. In the thymus-grafted mice the cells which were of bone-marrow derivation at this time can fairly be assumed to have entered the thymus graft, been somehow processed, and then shed to join and dilute the previously emergent cell population. Such processing and dilution could not

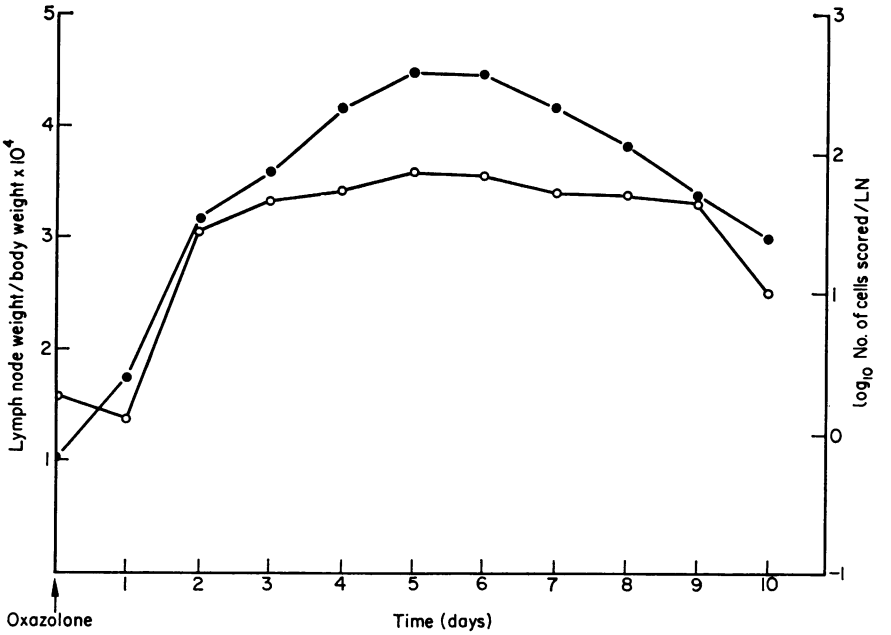

FIG. 10. A comparison of the changes in lymph node weight (●) and numbers of mitosing cells (○) in the lymph nodes in reconstituted mice (see text) during a 10-day period after painting with oxazolone.

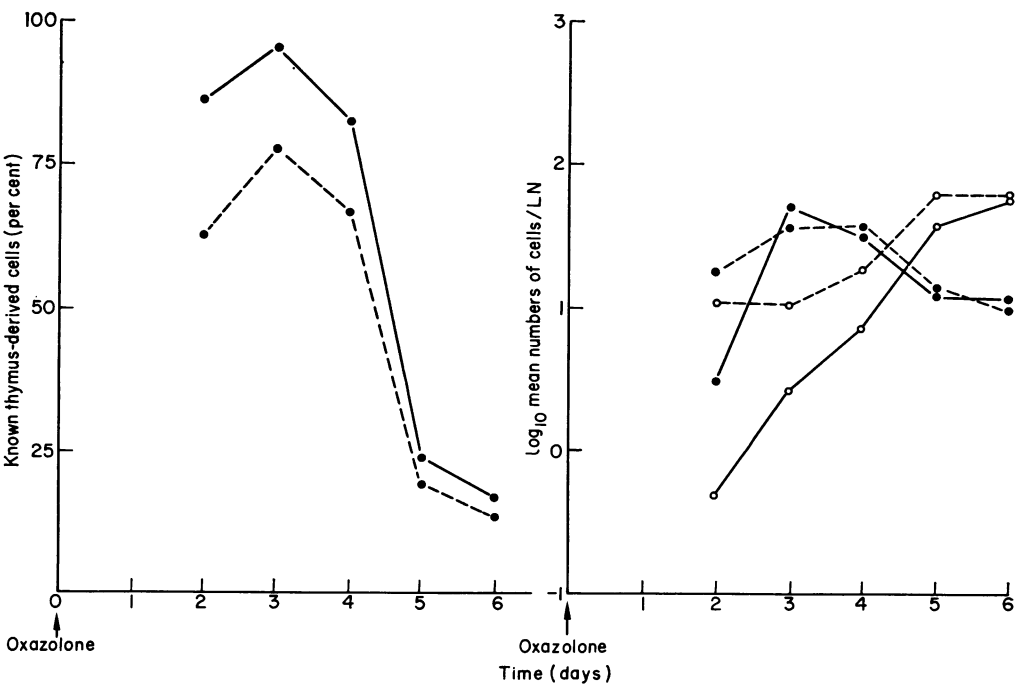

FIG. 11. The percentage and numbers of cells known to be of thymus (●) and bone marrow (○) origin in the draining lymph nodes of mice reconstituted with either thymocytes (——) or a thymus (– – –) graft (see text) during a period of from 2 to 6 days after painting with oxazolone.

occur in thymocyte reconstituted mice in which the necessary thymic epithelial framework did not exist. Further experiments (which will be fully reported elsewhere) have been made in which the thymus graft was removed 25, 30 or 50 days after irradiation and grafting and the cytological responses to oxazolone were tested at 70 days. From these unreported experiments it seemed that dilution of the known thymus-derived cells occurred between the 30th and 50th day.

The various results in this experiment can now be collated as follows. In *normal* adult CBA/H mice, oxazolone induces intense paracortical hyperplasia in the draining lymph nodes which begins on day 1, reaches a peak on days 3 and 4, and then declines. There is activation of the germinal centres and medullary cords which is first apparent on day 5; the response is maximal on day 8 and then slowly declines. In deprived mice, there is a negligible paracortical response to oxazolone; weak reactive changes develop in the germinal centres and medullary cords but these are short-lived and base-line appearances are regained by 10 days. *Reconstituted* mice, grafted with one lobe of CBA/H-*T*6*T*6 thymus, develop massive paracortical hyperplasia after exposure to oxazolone which is comparable to that observed in normal mice. Cytological analyses of nodes from reconstituted animals indicate that two cell-populations—thymus-derived and bone-marrow-derived—participate in the response. Thymus-derived cells proliferate first and follow a time-scale corresponding to the phase of paracortical hyperplasia. Bone-marrow-derived cells divide later, with maximal mitotic activity on days 5 and 6, and this increased proliferation is sustained. The period of heightened mitotic activity among bone-marrow derived cells coincides with the hyperplasia of germinal centres and medullary cords. The overall picture is presented in an idealized and semi-quantitative form in Fig. 12.

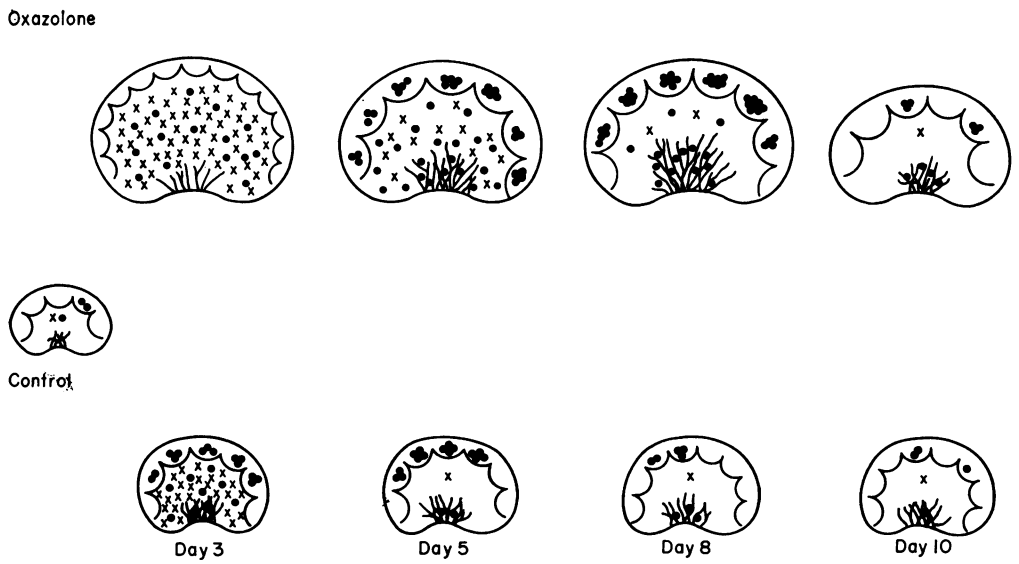

FIG. 12. A summary of the changes in the draining lymph nodes of reconstituted CBA mice (see text) during 10-days after either subcutaneous injection of sheep red blood cells or cutaneous painting with oxazolone. The sizes of the lymph nodes and the numbers of cells are accurate relative to each other but the actual location of the cells is based on histological appreciation. ×, Thymus-derived cell in mitosis; ●, bone-marrow-derived cell in mitosis. Most cells of bone-marrow origin in mitosis at day 3 are likely to have been processed by the thymus (see text).

DISCUSSION

Interest in the effect of oxazolone is largely due to its ability to produce a delayed hypersensitivity response, as shown in guinea-pigs by Turk (1967). It is, however, apparent that the studies which have been made in mice—including the present experiments—deal with the morphology of the primary response to oxazolone rather than with the *direct* measurement of the reaction evoked by a second contact with this substance. Although it is reasonable to suppose that the morphological changes bear some relationship to the phenomenon of delayed hypersensitivity, attempts to quantitate (or even detect) delayed hypersensitivity to topically applied antigens in mice have been largely unsuccessful. Asherson and Ptak (1968) have recently described a method based on measurements of ear thickness but one drawback of this procedure is that oxazolone seems to produce considerable non-specific swelling which may obscure the 'anamnestic' component of the response. This technical difficulty has for the moment delayed attempts to establish whether delayed hypersensitivity is 'thymus dependent' in the present experimental system.

The morphological responses produced by oxazolone in normal, deprived, and reconstituted mice have already been considered in some detail. One intriguing finding is that changes in the skin at the site of sensitization seem to be independent of the immunological status of the treated animal. In particular, the local reactions appeared to be unaffected by the absence of the thymus. Extensive epidermal necrosis occurred in all three experimental groups and it is possible that at least some of the cellular response in the regional lymph nodes was evoked by non-specific inflammatory changes in the adjacent skin. The observation that lymph nodes draining sites of application of croton oil may show paracortical activity is relevant here (Fjelde and Turk, 1965). Nevertheless, Waksman and his colleagues (Arnason, Jankovic, Waksman and Wennersten, 1962) have shown unequivocally in rats that delayed hypersensitivity responses are thymus-dependent, a finding which is consonant with all the previous findings of Turk, Parrott and their associates (see 'Introduction'). The present results confirm this view by showing that the principal cells which react in the early stages of the intense paracortical response to oxazolone are indeed of thymic origin.

Other conclusions from these experiments are less comfortable. Some of the previous studies of oxazolone have suggested that the response to this substance can be clearly distinguished from and contrasted with responses to other antigens which elicit little paracortical activity in the draining lymph nodes but cause prompt and considerable follicular hyperplasia with activation of germinal centres (Parrott and de Sousa, 1966). While such a distinction has considerable heuristic value, a striking feature of the present experiments is the sustained follicular response which emerged about 5 days after contact with oxazolone, a proliferative process largely associated with bone-marrow derived cells. It seems, then, that differences between the morphological response evoked by sheep red cells and by oxazolone may be quantitative rather than qualitative; both antigens appear to provoke consecutive proliferative activity of two cell populations in two distinct locations in the regional lymph nodes (Fig. 12). The response to sheep red cells has been further analysed in a number of different experimental systems (Claman, Chaperon and Triplett, 1966; Davies *et al.*, 1967; Mitchell and Miller, 1968) and all the results have suggested that thymus-derived cells do not themselves produce antibody but co-operate in some way with bone-marrow-derived cells in order that the latter may do so. It is not yet known to what extent proliferative activity among these two cell populations is a prerequisite for the

exercise of their separate functions and it cannot be said categorically that, because they show certain morphological similarities, the responses to oxazolone and to sheep cells are mediated by the same cell populations functioning in an analogous fashion. It may well be that the first portion of the response to oxazolone in mice is concerned with the development of a state of delayed hypersensitivity whereas the marked plasmocytic reaction which follows, and which is also largely thymus dependent, may be only relevant to the production of antibodies which are possibly not important for the hypersensitive state.

It remains to be seen, when an appropriate technique is developed, whether synergy can be demonstrated between thymus and bone-marrow derived cells in the events leading-up to the development of a hypersensitive immune state of the delayed kind or whether such an interraction is confined to antibody production.

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