A STUDY OF GLUCO-CORTICOSTEROID-INDUCED PYKNOSIS IN THE THYMUS AND LYMPH NODE OF THE ADRENALECTOMIZED RAT

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ABSTRACT

Pyknotic nuclei, observed in the thymus of steroid-treated rats, are dense, homogeneous, intensely basophilic and Feulgen positive. Under the electron microscope, the image is that of a complete segregation of the chromatin from the nuclear sap producing a margin or crescent of condensed chromatin. Approximately 30% of all small thymocytes appeared to undergo this type of degeneration within 3-4 hr after administration of the synthetic corticosteroid, dexamethasone. At this time, pyknotic thymocytes were observed in clusters, probably as a result of the activity of dense reticular cells and macrophages. Topographical and experimental data suggest the existence of a select population of steroid-sensitive thymic cells. Furthermore, on the basis of thymidine-3H incorporation studies, it appears that the steroid-sensitive population of thymocytes does not correspond to "aged" cells. In addition, many plasma cells became pyknotic after the same steroid treatment, indicating an unexpected similarity between their nuclei and those of lymphocytes. Finally, steroid failed to induce pyknosis of thymocytes in a variety of in vitro experiments, suggesting that the in vivo effect of steroid is of an indirect nature. The results are discussed in terms of (a) the nature of the nuclear changes characterizing pyknosis, (b) the hypothetical mechanism whereby steroids trigger such changes, and (c) the population of cells susceptible to steroid-induced pyknosis.

INTRODUCTION

A variety of stimuli, or "stresses", result in a sudden reduction in the size of the thymus. The phenomenon, first described by Dustin (1) as "choc caryoclasique", was later found by Selye (2) to be mediated via adrenocortical hormones. It is accompanied by nuclear pyknosis of small thymocytes (3, 4). Participation in lymphopoiesis and maintenance of a pool of immunologically competent cells appear to be the main functions of the thymus. However, the regulatory effects of glucocorticosteroid hormones on these two functions are not clear. Within recent years, experimental evidence has been obtained suggesting that some aspects of thymocyte function are related to features of thymus architecture. This concept has been substantiated by studies on the role of the thymus in: (a) the restoration of immunological competence in thymectomized animals (5), and (b) the induction of experimental leukemias (6, 7). Moreover, the level of mitotic activity of thymocyte appears to be influenced by the histological organization of the thymus (8). Nuclear pyknosis of thymocytes has not been investigated along these lines.

The first objective of this investigation is to examine, with the aid of the electron microscope, the
response of thymocytes to glucocorticosteroid hormones. The second is to provide evidence in support of the thesis that steroid-induced nuclear pyknosis in thymocytes is only expressed in vivo. The third is to evaluate whether steroid-induced nuclear pyknosis is restricted to lymphoid cells.

**MATERIALS AND METHODS**

**In Vivo Studies**

Male, Charles River Laboratory rats, 4 wk of age, were used in these studies. After bilateral adrenalectomy, the rats were maintained on 0.9% NaCl drinking water and food ad lib. Experiments were carried out 5–10 days after surgery. Completeness of adrenalectomy was checked upon autopsy of each animal.

Dexamethasone or dexamethasone-21-phosphate was injected intraperitoneally (Merck Sharp, & Dohme, West Point, Pa.) at a concentration of 7 mg/100 g body weight. Dexamethasone-21-phosphate was used as an aqueous preparation, while dexamethasone was dissolved in 75% dimethylformamide in water.

The rats were killed 1, 1.5, 2, 3, and 4 hr after administration of the hormone. Thymus, submandibular lymph node, spleen, and bone marrow were fixed in Karnovsky’s solution (9) and postfixed in buffered osmium tetroxide. The cytological effect of different doses of dexamethasone was also investigated (1.8 mg, 3.5 mg, 7 mg, and 14 mg/100 g body weight). For comparative purposes the effect of X-irradiation on the thymus was studied. Adrenalectomized rats, constrained within a lucite rig, were exposed for 3 min to 1500 R, delivered by an X-ray tube (Maxitron, General Electric 300; 300 kv/20 amps) and were examined 4 hr later. The half value layer of the beam was 2 mm of copper.

**In Vitro Studies**

Cell suspensions were prepared by mincing thymic tissue in Medium 199 (Hank’s Base) with glutamine, and incubating in an atmosphere of air at 37°C. The number of viable cells was determined on the basis of trypan blue exclusion. Approximately 90% of the cells were viable at the beginning of the experiment. 10^7 cells/ml were suspended in 5 ml of medium containing 10^{-3} M-10^{-4} M steroid and 15% isologous serum prepared from adrenalectomized rats. Control cell suspensions were incubated in the absence of steroid. After 4 hr of incubation, 75–80% of the cells were viable in control as well as experimental samples. The cells were centrifuged and fixed for 20 min in 1% buffered glutaraldehyde (10) followed by osmium tetroxide.

Thymus fragments, approximately 1 mm³, were incubated under similar conditions. They were fixed for 3 hr in Karnovsky’s solution followed by osmium tetroxide.

**Semi In Vitro Studies**

Rats received 7 mg/100 g of dexamethasone in vivo. Controls received the carrier solution. The animals were killed after 60–90 min, and thymus fragments were incubated for 2.5–3 hr in 5 ml of Medium 199 containing 15% rat serum and fixed in Karnovsky’s solution.

**Microscopy**

Sections for light microscopy were cut on an LKB ultramicrotome and stained with methylene blue (11) or according to the Feulgen method. For electron microscopy, thin sections were stained with 4% uranyl acetate in methanol (12) followed by lead citrate (13), and examined in a Siemens Elmiskop I electron microscope equipped with a 50 µ molybdenum objective aperture and operated at 80 kv.

**Radioautography**

1) **THYMIDINE^{3H} IN VIVO FOLLOWED BY DEXAMETHASONE IN VIVO:** Two rats received 0.40 mCi of thymidine^{3H} (SA 12.0 Ci/m mole; Schwarz Bio Research Inc., Orangeburg, N. Y.) per 100 g body weight, intravenously (tail vein). After 1 hr, one received 7 mg of dexamethasone per 100 g body weight, intraperitoneally, and was killed 4 hr later. The second received 7 mg of dexamethasone per 100 g body weight, 24 hr after the injection of thymidine^{3H}, and was killed 4 hr later. In both cases the thymus was removed immediately and immediately fixed.

2) **DEXAMETHASONE IN VIVO FOLLOWED BY URIDINE^{3H} IN VITRO:** Dexamethasone was injected intraperitoneally. 4 hr later, a thymus cell suspension was prepared in 5 ml of Medium 199 at room temperature containing 25 µCi/ml of 5-uridine^{3H} (SA 20 Ci/m mole; 13 X 10^{-2} M; Schwarz Bio Research Inc.). Approximately 10^7 cell/ml were incubated for 1 hr at 37°C. The cells were washed and immediately fixed as previously described.

The procedure of Caro and van Tubergen (14) was followed for both light and electron microscope radioautography. The sections were stained with methylene blue for light microscope radioautography and with 4% uranyl acetate followed by lead citrate for electron microscope radioautography.

**RESULTS**

**Controls**

Pyknosis is physiologically induced by corticosteroid hormones in small lymphocytes and thymo-
cytes. On the other hand, laboratory animals are subjected to a variety of "stresses" resulting in increased adrenocortical secretion. It follows that normal rats, for example, are poor controls in a study of experimentally induced pyknosis, while adrenalectomized ones are more suitable for this purpose. Indeed, our observations confirm that the pyknotic index is considerably lower in adrenalectomized rats. It was therefore decided to use adrenalectomized rats to avoid unpredictable interference from endogenous corticosteroid hormones.

The Pyknotic Nucleus

LIGHT MICROSCOPY

Pyknotic nuclei are round, dense, homogeneous, intensely basophilic, and Feulgen positive.

ELECTRON MICROSCOPY

In normal interphase thymus nuclei a clear separation between the dense margined heterochromatin and the central euchromatin is observed. When pyknosis occurs, there is complete segregation of the chromatin from the nuclear sap; the chromatin becomes margined and assumes the condensed configuration of heterochromatin, while a small interior area remains electron-lucent. This clear zone often contains a number of electron-opaque granules similar to the "dense body" described in pyknotic lymphoma cells (15).

Frequently, the condensed part of the pyknotic nucleus appears as a characteristic crescent (Fig. 1), sharply delineated from the remaining clear zone. However, this image is not observed in all pyknotic cells, probably because of the random plane of sectioning. Although serial sections were not made, we favor the interpretation that all pyknotic nuclei show similar segregation between chromatin and nuclear sap. (Since margined chromatin is normally more conspicuous after aldehyde fixation, a few samples were examined after osmium fixation alone, and these too showed the same pattern of chromatin segregation).

Under the electron microscope the chromatin of pyknotic nuclei has a density similar to or greater than that of heterochromatin, while the clear zone lacks the fine fibrillar structure of euchromatin. Under the light microscope it is Feulgen positive and can easily be recognized in 1 µm sections, while the clear zone shows only a faint reaction. Radioautography, after thymidine-3H incorporation, shows grains overlying the dense crescent almost exclusively.

An over-all reduction in nuclear size accompanies pyknosis. Small thymocyte nuclei average 4.5–6.1 µ in diameter; after they become pyknotic they average only 2.9–4.5 µ (Table I). Finally, as seen in Table II, pyknotic nuclei showed a significantly decreased incorporation of 5-uridine-3H, indicating that as far as RNA synthesis is concerned, the metabolism of condensed chromatin is markedly reduced.

Time Study

In previous light and electron microscopy studies on pyknosis (38, 39), investigators have focused their attention on late phases of pyknotic degeneration (up to 24 hr). Our electron microscope study indicates that earlier stages can easily be identified. Pyknotic indexes, at various times after dexamethasone, are presented in Table III. At 1 hr the pyknotic index is similar to that of adrenalectomized controls. However, 8% of the small thymocytes were pyknotic by 2 hr, 23% by 3 hr, and 30% by 4 hr. Under the conditions of our experiments, it seems that all of the small thymocytes susceptible to steroid-induced pyknosis undergo this type of degeneration within 3–4 hr after administration of dexamethasone. Unfortunately, it was difficult to confirm this point by studying later stages because a considerable degree of karyorrhexis appears by 6-8 hr after treatment and makes the determination of the pyknotic index meaningless. It appears, therefore, that the sensitivity to steroid-induced pyknosis is restricted to a finite population of small thymocytes. However, an alternate interpretation is that by 4 hr the plasma level of active steroid has decreased below that which is required to induce pyknosis.

Dose Response

If, 4 hr after treatment with dexamethasone, pyknosis decreased as a result of a rapid catabolism of the steroid, one could expect a higher dose to affect a greater number of cells. This, however, has not been observed in the experiment summarized in Table IV. Doses as high as 14 mg/100 g body weight failed to increase the pyknotic index. Again, the results suggest the existence of a select population of steroid-sensitive thymic cells.
Figure 1 Thymus of adrenalectomized rat 4 hr after dexamethasone. Several thymocytes show the typical chromatin condensation corresponding to pyknosis (P). In one case the plane of section demonstrates a chromatin crescent (arrow). Other pyknotic nuclei are already within the cytoplasm of phagocytic dense reticular cells (P'). X 6800.
TABLE I

Diameters of Nuclei In Thymocytes after Dexamethasone Treatment

| Experiment Number | Pyknotic* | Normal |
|-------------------|-----------|--------|
| 37                | 4.0       | 5.6    |
|                   | 3.2       | 6.1    |
|                   | 3.5       | 5.0    |
| 41                | 2.9       | 4.8    |
|                   | 4.4       | 4.5    |
|                   | 3.7       | 3.9    |
|                   | 4.5       | 4.7    |
| 45                | 3.2       | 5.2    |
|                   | 3.6       | 5.0    |
|                   | 3.9       | 5.8    |
|                   | 3.7       | 5.5    |
| 58                | 3.8       | 4.8    |
| Average           | 3.7       | 5.1    |

* In those cases where the nucleus did not approximate a sphere the geometric mean was substituted for the diameter.

Topography

2 hr after steroid treatment, pyknotic cells appear randomly distributed throughout the thymus cortex. At this time, no foci or clusters of pyknotic cells are observed.

As described in a previous study (16), fixation with Karnovsky's solution (9) at room temperature preserves the intimate contact between thymocytes and reticular cells. Slender cytoplasmic processes of a particular class of reticular cells extend out between small thymocytes, frequently separating thymocytes from each other. These dendritic-like reticular cells have a very dense cytoplasmic matrix and are referred to as "dense reticular cells" (DRC) (16). They occasionally show signs of phagocytic activity.

Our observations confirm the presence of DRC's (Fig. 2). Thymocytes are frequently surrounded by the cytoplasmic processes of these reticular cells which could, therefore, control the transfer of substances such as steroids. The same reticular cells, however, participate in the phagocytosis of degenerating thymocytes as considered in the next section.

TABLE II

Thymocyte Incorporation of 5-Uridine-3H In Vitro after Dexamethasone In Vivo

| Field Number | Pyknotic Thymocyte | Normal Thymocyte |
|--------------|--------------------|------------------|
| 1            | 2.0                | 6.9              |
| 2            | 2.0                | 7.4              |
| 3            | 2.8                | 7.5              |
| 4            | 1.5                | 7.5              |
| 5            | 1.6                | 9.5              |
| 6            | 1.7                | 9.6              |
| 7            | 1.5                | 8.9              |
| 8            | 1.8                | 7.9              |
| 9            | 2.0                | 6.4              |
| 10           | 1.8                | 9.5              |
| 11           | 2.6                | 7.9              |
| 12           | 1.8                | 10.9             |
| 13           | 2.8                | 9.8              |
| 14           | 1.5                | 10.1             |
| 15           | 2.3                | 9.0              |
| 16           | 2.0                | 10.8             |
| 17           | 1.6                | 9.5              |
| 18           | 0.8                | 9.5              |
| 19           | 1.4                | 8.5              |
| 20           | 2.0                | 7.8              |

Median 2.0 8.8

Average Grain Count

TABLE III

Time Study of the Incidence of Pyknosis after Dexamethasone Treatment

| Time | Pyknosis* |
|------|-----------|
| 0    | %         |
| 1    | 2         |
| 2    | 8         |
| 3    | 25        |
| 4    | 30        |

* At least 1000 cells were counted. Counts were made from photographs. % pyknosis, number of pyknotic nuclei/number of normal nuclei X 100.

Phagocytosis

The random distribution of pyknotic thymocytes observed at 2 hr is replaced by the formation of foci or clusters of pyknotic cells, visible at 3 and 4 hr (Fig. 3). The clustering of pyknotic thymocytes probably results from the engulfment and progressive digestion of several degenerating cells within macrophages. Whether or not all these...
TABLE IV
Incidence of Pyknosis after Various Doses of Dexamethasone

| mg dexamethasone/100 g body weight | %     |
|-----------------------------------|-------|
| 1.8                               | 34    |
| 3.5                               | 27    |
| 7.0                               | 27    |
| 14.0                              | 36    |

*mg dexamethasone/100 g body weight.

The frequent finding of pyknotic nuclei within the cytoplasm of a typical DRC, and of the engulfment of pyknotic thymocytes by their cytoplasmic processes, suggests that DRC's initiate the process of phagocytosis of pyknotic cells. Fragmentation of pyknotic nuclei (karyorrhexis) as well as cytoplasmic shedding are secondary phenomena occurring within the macrophages. The initial visible alterations within small thymocytes are restricted to their nuclei and occur as early as 2 hr after steroid treatment when cytoplasmic alterations are not yet observed and the plasmalemma is still intact.

A Steroid-Sensitive Population of Cells

In an attempt to identify the population of steroid-sensitive cells, experiments were conducted (a) to evaluate the "cell age" of the sensitive thymic cells, (b) to study nonthymic cells after steroid treatment, and (c) to compare the effects of steroid with those of X-irradiation.

(a) Under normal circumstances a large number of small thymocytes are believed to be "end cells", surviving for a considerable length of time without undergoing mitosis. Could the population of steroid-sensitive thymocytes correspond to the oldest cells? To put the hypothesis to a test, cell age, i.e. time elapsed since a last S phase, was evaluated in terms of thymidine-3H incorporation by counting the number of labeled pyknotic cells. Two samples were compared; both received dexamethasone 4 hr before sacrifice. In one case, the rat was treated with thymidine-3H 1 hr before steroid administration while in the other, treatment was 24 hr earlier. If the administration of steroid accelerates a normal aging process, one would not expect labeled pyknotic cells in these two short term thymidine-3H incorporation experiments. However, labeled pyknotic cells were found in both. The rate at which small thymocytes are labeled is a logarithmic function of time (17). It was therefore anticipated that a higher proportion of labeled pyknotic thymocytes would be found at 24 hr than at 1 hr after labeling. However, in both cases a similar proportion of pyknotic cells was labeled (13% after 1 hr; 19% after 24 hr, Table V). These results suggest that steroids induce pyknosis in small thymocytes which have recently incorporated thymidine-3H as well as in those that have not. Therefore, it appears unlikely that the steroid-sensitive population of thymocytes corresponds to aged cells.

(b) To further characterize the population of steroid-sensitive cells, samples from other organs were studied: lymph nodes, spleen, bone marrow, liver, and intestine.

4 hr after dexamethasone, the germinal centers of lymph nodes contained pyknotic nuclei, mostly within macrophages, and were surrounded by a corona of tightly packed, normal small lymphocytes. Spleen and bone marrow cells also showed various degrees of pyknosis after dexamethasone. Hepatic and intestinal epithelial cells, however, never showed this type of nuclear degeneration (Table VI). It seemed, therefore, that in accordance with previous studies (4), the steroid-induced condensation of chromatin was restricted to thymic or lymphatic lymphocytes. However, a careful study of submandibular lymph nodes led to a surprising observation: 4 hr after dexamethasone, many typical plasma cells were also pyknotic (Fig. 4). In several cases the plasma cells still had an easily recognizable cytoplasm (Fig. 6, 7) while in others, the cells were in more advanced stages of degeneration, within macrophages. Crescents of condensed chromatin seen in plasma cells appeared identical to those seen in thymocytes. Under the light microscope, pyknotic plasma cells often showed a "foamy" cytoplasm (Fig. 5) which ultrastructurally corresponded to the cisternae of the rough endoplasmic reticulum.

(c) The nuclear alterations induced by steroids were indistinguishable from those induced by X-irradiation: 4 hr after supralethal X-irradiation of adrenalectomized rats the number and distribution of pyknotic cells was similar to that observed after steroid treatment.

**In Vitro Studies**

How direct is the effect of dexamethasone on thymocyte nuclei, and is the injected hormone or...
FIGURE 2 Thymus of a steroid-treated adrenalectomized rat. No pyknosis is observed in this field which illustrates, however, a typical dense reticular cell (DRC) and its slender cytoplasmic extensions frequently preventing direct contact between the thymocytes (arrows). × 11,000.
Figure 3  Thymus of adrenalectomized rat 4 hr after dexamethasone. Pyknotic cells, within the cytoplasin of a DRC, are clustering around a macrophage (M). One pyknotic thymocyte (D) shows a degenerating cytoplasm. Note the good preservation of intimate cell contacts. The dense cytoplasm of reticular cells is recognizable in many places (arrows).  $\times$ 6800.
TABLE V
Incidence of Pyknosis 4 Hr after Dexamethasone in Rats 1 Hr or 24 Hr Previously Pretreated with Thymidine-3H

| Thymidine-3H | P-L/pyknotic* | P-L/labeled† |
|--------------|---------------|--------------|
| hr           | %             | %            |
| 1            | 3.1           | 13           |
| 24           | 23.0          | 19           |

* P-L/pyknotic, number of nuclei which were pyknotic and labeled/number of pyknotic cells × 100.
† P-L/labeled, number of nuclei which were pyknotic and labeled/number of labeled cells × 100.

TABLE VI
Incidence of Pyknosis After Dexamethasone Compared in Various Tissues

| Tissue                  | Adrenal-ectomized control | 4 Hr after dexamethasone |
|-------------------------|----------------------------|--------------------------|
| Thymus                  |                            |                          |
| Cortex                  | ±                          | +                        |
| Medulla                 | ±                          | +                        |
| Lymph node              |                            |                          |
| Germinal center         | +                          | +                        |
| Corona                  | −                          | −                        |
| Medulla-plasma cell     | ±                          | +                        |
| Spleen                  |                            |                          |
| Germinal center         | +                          | +                        |
| Corona                  | −                          | −                        |
| Red pulp-plasma cell    | ±                          | +                        |
| Bone Marrow             |                            | +                        |
| Intestine               |                            |                          |
| Crypt                   | −                          | −                        |
| Villus                  | −                          | −                        |
| Liver                   | −                          | −                        |

Suspensions of Small Thymocytes

Despite the fact that the thymic cells and the serum were both prepared from adrenalectomized rats, suspensions of these untreated thymocytes showed a significant incidence of pyknosis. Various modifications of the incubation medium failed to provide better controls. It is believed that pyknosis is a general degenerative phenomenon, triggered by factors other than corticosteroid hormones, and that in vitro manipulations can lead to the same alterations. This observation, however, made our attempts to reproduce the effect of steroid in vitro difficult and of questionable significance. Moreover, the addition of 10⁻⁴ M of dexamethasone failed to induce any significant increase in the pyknotic index (Table VII).

Incubated Thymus Fragments

Small thymus fragments from adrenalectomized rats were incubated under conditions similar to those used for thymocyte suspensions. The addition of dexamethasone also failed to induce a significant increase in the pyknotic index.

Semi-In Vitro Observation

When thymus fragments were prepared 1–1.5 hr after the in vivo administration of the hormone, and then incubated for an additional 2.5–3 hr, no increase in pyknosis was observed, in sharp contrast with the results of all of our in vivo observations.

Discussion

The discussion will be limited to three problems: (a) the nature of the nuclear changes characterizing pyknosis, (b) the hypothetical mechanisms whereby steroids trigger such changes, and (c) the population of cells susceptible to steroid-induced pyknosis.

The chromatin of the interphase nucleus is considered to exist in two distinct forms: heterochromatin and euchromatin. The marginalized heterochromatin and the loose meshwork of euchromatin are clearly recognized under the electron microscope. Radioautography has demonstrated that euchromatin is active in RNA (18) and also in DNA synthesis (18, 19). The fibrillar structures of heterochromatin are believed to be continuous with those of euchromatin (20), and the interphase nucleus might therefore be described in terms of an equilibrium between the metabolically active euchromatin and the dormant hetero-
FIGURE 4  Submandibular lymph node of adrenalectomized rat 4 hr after dexamethasone. Four plasma cells are seen, one undergoing pyknosis (P). The complete margination of the chromatin is identical to what has been observed in thymocytes and lymphocytes under the same experimental conditions. The pyknotic plasma cell still has an unaltered cytoplasm. × 11,000.

FIGURE 5  Light micrograph of the lymph node seen in Fig. 4. One pyknotic plasma cell shows a “foamy” cytoplasm most likely corresponding to the cisternae of the endoplasmic reticulum (A). Another one shows the typical crescent condensation of the chromatin (B). Other pyknotic nuclei are already within a macrophage (C). × 2200.
chromatin. The equilibrium is apparently shifted under a variety of physiological as well as experimental conditions. For example, the differentiation of normoblasts into reticulocytes is accompanied by a progressive chromatin condensation. On the other hand, transformation of small lymphocytes by phytohemagglutinin exemplifies how chromatin can revert to a metabolically active state with the concomitant dispersion of most of the condensed heterochromatin.

An ultrastructural similarity exists between the chromatin crescent of pyknotic nuclei and the marginated heterochromatin of intact cells. Heterochromatin is always observed in close contact with the nuclear envelope. Similarly, the condensed chromatin of pyknotic nuclei is invariably marginated, in intimate contact with the nuclear envelope. This is in agreement with recent observations showing that the interphase chromatin fibers are permanently attached to the nuclear envelope.

In view of these considerations, it seems appropriate to formulate the following hypothesis: corticosteroid hormones, administered in vivo, may shift the equilibrium between euchromatin and heterochromatin in a limited number of susceptible lymphoid cells. This would result in a complete and irreversible condensation of the nuclear chromatin accompanied by a decreased metabolic activity as illustrated by the reported reduction in 5-uridine-3H incorporation.

It is of interest to note that an antagonism has been observed between cortisol and phytohemagglutinin in in vitro experiments dealing with the activation of small lymphocytes. However, these observations are difficult to compare with ours since steroids failed to produce pyknosis in several in vitro systems.

Biochemical methods will be necessary to elucidate the fundamental mechanisms involved in steroid-induced pyknosis. However, our ultrastructural observations give support to the concept that corticosteroids induce pyknosis in thymocytes by an indirect mechanism. Indeed, it has been reported that essentially all of the steroid hormone enters and leaves the thymus in about 15 min. However, small thymocytes do not appear pyknotic until 2 hr after treatment in vivo, suggesting a complex chain of metabolic events. Thymic cell suspensions or fragments of thymus tissue incubated with high concentrations of corticosteroid hormones showed no significant increase in the number of pyknotic cells. In thymus fragments one can assume that cell-to-cell relationships, and more precisely the microenvironment of small thymocytes created by reticular cells (DRC's), are preserved. The fact, however, that thymus fragments incubated with the hormone failed to show a significant increase in the number of pyknotic cells seems to rule out a prime and exclusive role of the dense reticular cells in the induction of the phenomenon. Steroid-induced pyknosis appears, therefore, to result from a complex mechanism in which extra-thymic metabolic events are probably

| Steroid                          | Pyknosis* | Block 1 | Block 2 | Block 3 |
|----------------------------------|-----------|---------|---------|---------|
|                                  | %         | I       | II      | I       | II      | I       | II      |
| Dexamethasone phosphate (10^-4 M) | 8 6       | 10 1    | 3 15    |
| Dexamethasone dissolved in dimethyl formamide (10^-4 M) | 14 12 | 2 3 | 4 5 |
| Controls                         |           |         |         |         |
| Untreated                        | 2 1 4 10 9 13 |
| Dimethylformamide                | 13 7 2 3 8 6 |

* % of pyknosis, number of pyknotic nuclei/number of normal nuclei X 100.
‡ I and II refer to different fields of the same slide.

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FIGURES 6-7 Submandibular lymph node of adrenalectomized rat 4 hr after dexamethasone. Two typical plasma cells showing the characteristic chromatin condensation of nuclear pyknosis. No visible alterations are recognizable within their cytoplasm. Fig. 6, X 14,000. Fig. 7, X 20,000.
involved. Macrophages and dense reticular cells do not appear instrumental in the induction of thymic pyknosis, but they do, however, participate actively in the disposal of pyknotic thymocytes. Both cells participate in cluster formation, phagocytosis, and lysis of pyknotic thymocytes. 4 hr after in vivo treatment with steroid the irreversible nature of the process is indicated by the presence of most of the pyknotic thymocytes within macrophages. How can macrophages recognize pyknotic thymocytes when they still possess intact plasma leafma and unaltered cytoplasm? We can only hypothesize as to the nature of the signals received by the macrophages to initiate this action. A variation in surface charge or a sudden loss of mobility of the pyknotic cell might be critical factors. However, neither of these changes can be demonstrated by electron microscopy of fixed material.

The methods employed in this study provide a comprehensive cytological characterization of steroid-sensitive cells. Only 30% of the small thymocytes undergo pyknosis after a large single dose of dexamethasone. Steroid-sensitive thymocytes are not aged cells, as indicated by our thymidine-3H experiments. Although clusters were scattered in a random fashion throughout the thymus cortex, they were frequently observed in close proximity to cells in mitosis. This finding may exemplify a mitosis-stimulating effect of degenerated lymphocytes which has been previously reported (25). As estimated by their number and distribution, the population of steroid-sensitive thymocytes resembles that of X-irradiation-sensitive cells. In these experiments the animals were adrenalectomized and the effects of the irradiation can not therefore be due to a sudden release of adrenocortical hormones.

The population of steroid-sensitive cells is not limited to the thymus. Small thymic and lymphatic lymphocytes are sensitive to corticosteroid (3). Could this phenomenon be analyzed in terms of “short-lived” versus “long-lived” lymphocytes? The short-lived variety is found in the thymus, germinal centers, and bone marrow. According to several authors (26, 27, 28), the short-lived variety is steroid-sensitive while the long-lived cells are not. Short-lived lymphocytes present in germinal centers were also found to be steroid-sensitive while long-lived ones present in the corona were not (29). However, in the thymus 95% of the thymocytes belong to the short-lived variety (30, 31), while in our experience only ±30% undergo pyknosis 4 hr after dexamethasone. As suggested by Everett and Tyler (32), functional considerations most likely will further subdivide the class of short-lived lymphocytes.

Plasma cells also undergo pyknosis after steroid treatment. This observation was unexpected and its implications are not clear. Plasma cells are known to synthesize antibody (33, 34), and it is tempting to relate our observations with the immunosuppressive effects of corticosteroid hormones. However, the steroid’s effectiveness appears to depend upon the length of time of its administration before challenge with antigens (35, 36). Steroid therapy does not affect established antibody production. Present techniques of electron microscopy are not likely to contribute more insight unless ultrastructural features are described which would enable us to distinguish between the different classes of lymphocytes and plasma cells. Nevertheless, our observations give new support to the findings of Gowans and McGregor (37) that plasma cells may derive from small lymphocytes via a large pyroninophilic cell intermediate, in view of the fact that the nuclei of lymphocytes and plasma cells share a peculiar sensitivity to steroid-induced pyknosis.

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