fects reported for intrasynovially administered insoluble glucocorticoids(5). Two counterirritants applied topically to the arthritic knee before, during and after urate were inactive; but in preliminary tests the anthranilic acid derivative CI-583(6) prevented the inflammatory response. Within the doses of urate studied (4 and 10 mg), decreasing the dose of urate did not affect the response to either phenylbutazone or indomethacin. The results indicate that this procedure may be a useful laboratory test for oral screening and evaluation of nonsteroidal anti-inflammatory, antiarthritic agents.

Summary. Oral administration of the nonsteroidal anti-inflammatory agents phenylbutazone and indomethacin prevented or reversed a urate-induced synovitis in unanesthetized dogs. Acetylsalicylic acid, codeine, promazine, chlorpromazine, topical counterirritants and intravenous hydrocortisone were inactive. This model of gouty arthritis may be useful in detecting and evaluating orally active nonsteroidal anti-inflammatory agents.

Appreciation is expressed to Merck Sharp & Dohme for indomethacin; to Geigy Pharmaceuticals for phenylbutazone; and to Parke, Davis & Co. for CI-583.

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The Autofluorescent Cells of the Rat Thymus Under Certain Experimental Conditions.* (31230)

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In a previous work of fluorescence microscopy(1), we reported the presence of peculiar "autofluorescent cells" at the cortico-medullary junction of the young adult rat thymus (Fig. 1). The cytoplasm was filled with granules most of which were small and yellow autofluorescent. These granules which were not detectable at birth, and first became evident between days 15 and 20 and increased in number until about the sixth month(1,2). The granules which stained variably with the techniques of Sudan black(1,3) and P.A. Schiff(1,2), were thought to contain lipids(1, 3).

Because it was thought that the autofluorescent cells were absent in the thymus prior to its involution, it has been proposed that these cells are an expression of altered thymic metabolism accompanying thymic involution(3). It was later suggested that the origin of these cells might be related to lympholysis as their granules could represent indigestible residues from lysis of lymphocytes by macrophages(4). We previously suggested that these cells are involved in immunity or growth(1,5).

The first purpose of this work, to determine if the lipoid granules of the autofluorescent cells have an endogenous origin, was investi-

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gated by placing weaned rats on a fat-free diet. A second purpose, to correlate the formation of these cells with thymic involution, was studied by treatments slowing this involution. The third purpose, to find out if these cells constitute a reserve of mobilizable macrophages, was investigated by provoking thymic lympholysis and, consequently, an intense macrophagic activity.

Materials and methods. Histology. In all cases, the rats were killed by chloroform. Their thymi were fixed in cold alcohol and embedded in paraffin as described elsewhere (1). For fluorescence microscopy, unstained sections were examined using previously reported conditions(1). Some sections were stained by the technique of Dominici (eosin Y-orange G and toluidine blue) for bright field microscopy.

Origin of autofluorescent granules. Six male and six female weaned Sprague-Dawley rats aged 3 weeks were placed on an absolute fat-free diet of the following composition:

Dextrose	71 g
Casein (vitamin-free)	20 "
Celluloflour	5 "
Salt mixture (U.S.A. XIV)	4 "

This diet was available to the animals *ad libitum* until they were killed at the age of 10 weeks.

Slowing of thymic involution. Five-week-old Sprague-Dawley rats were treated as follows: 6 were orchiectomized, 6 male and 6 female were adrenalectomized and placed on saline. These rats were killed when 3 months old.

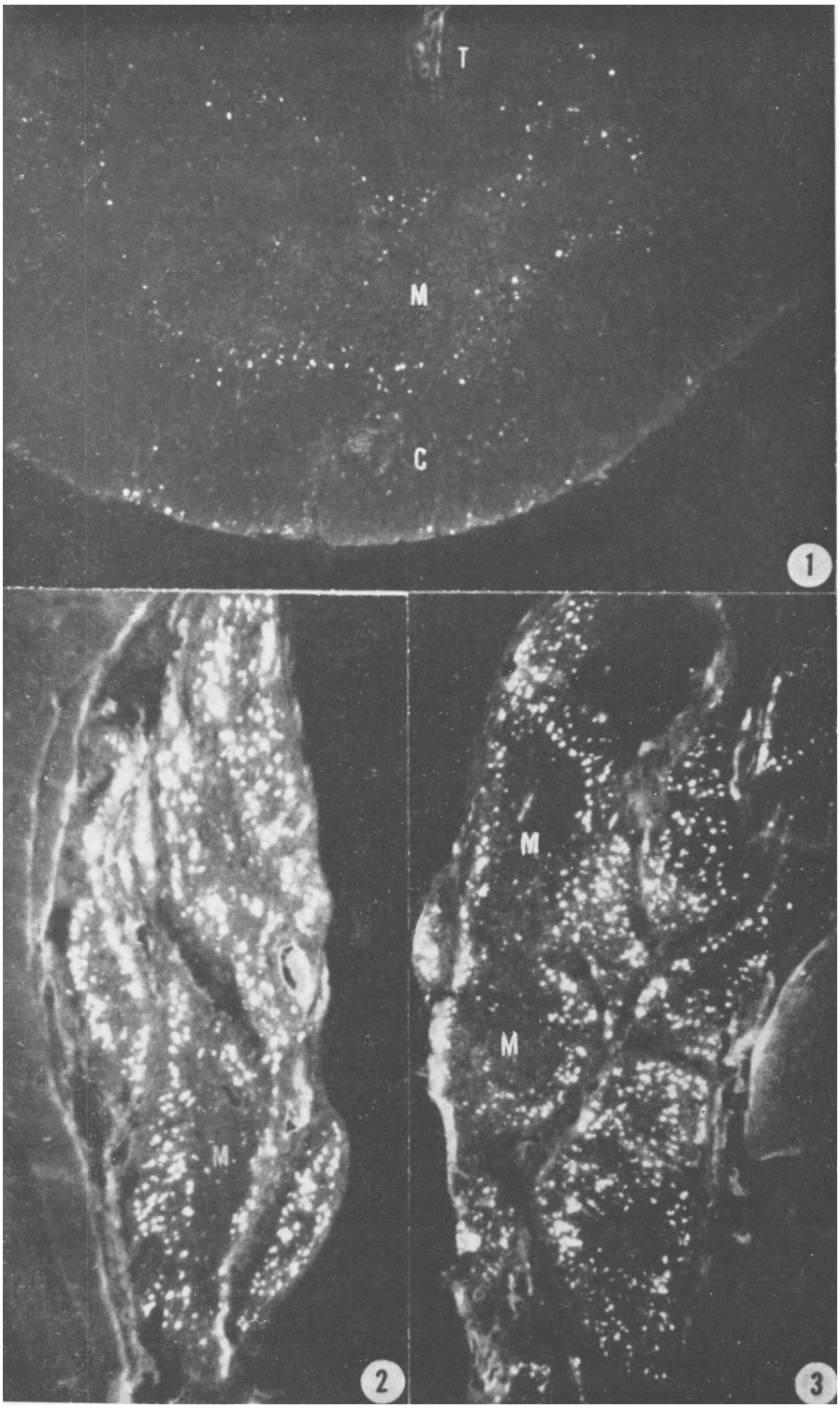
Thymic lympholysis. Each of 20 female Sprague-Dawley rats, 2 months old, was given an intraperitoneal injection of 20 mg of cortisone acetate. A similar group of rats was irradiated over the thymus with a dose of 600 r.‡ Five rats of each group were killed on days 1, 2, 4 and 6 after treatments.

Results. Origin of autofluorescent granules. After 7 weeks of an absolute fat-free diet, the rats were half as large as normal rats of the same age, and had little thymus left (compare Fig. 1-3). The involuted thymi consisted of small medullary areas surrounded by a very thin layer of cortex. The medulla was devoid of autofluorescent cells while in the cortex (Fig. 2-4), the autofluorescent cells were concentrated along the corticomedullary junction as in normal rats. In addition, some of these cells were present throughout the cortex and often covered about half of the surface of the remaining cortex. Hence, in these experimental rats, the concentration of autofluorescent cells per unit area of cortex was much greater than normal. Moreover, many of these cells appeared to be larger and brighter than normal. Whether they were actually larger or represented a few adjacent normal sized cells cannot be determined as the outline of these cells was hardly detectable. Examination of the same unstained section under bright field microscopy revealed the presence in many autofluorescent cells of yellow pigment which was barely detectable in the normal thymus(1). In sections stained by the technique of Dominici, mainly small lymphocytes were seen in addition to the autofluorescent cells. Lymphocytic mitoses were rare. Occasionally, small pycnotic masses were present in the autofluorescent cells.

Slowing of thymic involution. At 3 months, the adrenalectomized and the orchiectomized rats had thymi of maximal size. In these thymi, the distribution and the amount of autofluorescent cells appeared to be as in the differentially involuted thymi of normal rats of the same age.

Thymic lympholysis. The irradiated thymi had a much thinner cortex than normal thymi of the same age. This resulted from an intense lymphocytic degeneration as observed in the Dominici stained sections. During the 6 days following irradiation, the appearance and the pattern of distribution of the autofluorescent cells seemed to be that of normal thymi. Throughout the cortex, however, there appeared to be a slightly larger amount of scattered yellow autofluorescent granules than in corresponding normal thymi. The results for the rats injected with cortisone resembled those for irradiated rats, except for the following: during the first 2 days after cortisone injection, the autofluorescent cells appeared

‡ Irradiations were performed by Mr. J. Kruuv of the Victoria Hospital, London, Ontario, to whom we express our thanks.


T
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The 3 Figures were taken under a fluorescent microscope from unstained sections of thymi fixed in cold alcohol and embedded in paraffin. Bright autofluorescent cells appear white in the Figures. Magnification about × 60.

FIG. 1. A thymic lobule from a normal 10-week-old rat shows a row of scattered autofluorescent cells outlining its cortico-medullary junction. Medulla (M) and cortex (C) are nearly devoid of these cells. The T is at right of a small portion of a trabecula.

FIG. 2 and 3. Each Figure shows a considerably involuted thymic lobe (formed of several lobules) from 2 ten-week-old rats placed on a fat-free diet at the age of 3 weeks. Each of the few small areas nearly devoid of autofluorescent cells represents the medulla (M) of a lobule. The cortical layer of each lobule is very thin and about half filled with very bright autofluorescent cells, many of which appear larger than in a normal thymus of the same age (Fig. 1).

to us to contain fewer and paler yellow autofluorescent granules than normally. Later, the appearance of these cells was normal again.

Discussion. Adrenalectomy and orchiectomy are known to slow the process of thymic involution(6). The maximal size of the thymi of the experimental rats even suggested that thymic involution had not yet started in these animals. The similarity between the pattern of autofluorescent cells in these thymi and that in the differentially involuted thymi of unoperated rats of the same age demonstrates that the development of the autofluorescent cells is not linked to thymic involution. These cells are thus an expression of a normal thymic activity occurring even before involution.

The results for the rats placed on a fat-free diet, at an age when the granules of the thymic autofluorescent cells are just starting to appear, reveal the endogenous origin of the lipids in these granules. In the thymi of these rats, the autofluorescent cells were much more abundant, per unit area of cortex, than normal. This resulted from the fact that, while the absolute number of these cells appeared to be as great as normally, the thymic volume was considerably decreased. Food and vitamin restrictions are indeed known to cause a severe thymic involution(6). Although this cannot be ascertained here, it is possible that the absolute number of these cells was even greater than normal. By contrast, other thymic cell types, particularly the large and medium lymphocytes, were considerably fewer than normal. This contrast suggests, first, that the autofluorescent cells develop independently of lymphocytopoiesis and, consequently, of lympholysis because the intensity of both processes is known to be considerably decreased during prolonged inanition(6). Secondly, it suggests that the lipoid granules of these cells represent the product of a synthetic activity rather than an accumulation of residual products from thymic metabolism. Indeed, if the latter case were true, one would expect the autofluorescent cells and their granules to be fewer than normal, since most thymic activities were much more decreased after weaning, *i.e.,* a time when granules are hardly detectable in the thymus. Whether or not the lipids of these cells are essential to growth remains to be determined. Finally, as a chance observation, we might further report that the parenchymal cells of the liver and kidneys from the rats suffering inanition contained as many yellow autofluorescent granules as observed previously in normal tissues(5).

In irradiated thymi and in thymi of cortisone-treated animals, massive lympholysis occurs which causes an intense macrophagic activity in the thymic cortex during the next 2 days(7-9). During that period, the autofluorescent cells remained located at the cortico-medullary junction while the cells of the cortex undergoing an intense phagocytosis were not autofluorescent, although some contained a few autofluorescent granules. This observation indicates that the autofluorescent cells do not constitute a reserve of mobilizable genuine macrophages. This is in agreement with our previous conclusion(1) made on the basis of other observations.

Summary. Autofluorescent cells, having abundant lipoid granules, were previously found at the cortico-medullary junction in the thymi of 10-week-old rats. In this investigation, rats were treated variously to test possibilities concerning the undetermined nature of these cells. First, rats aged 5 weeks were adrenalectomized or orchiectomized and killed when aged 3 months. In their non-involuted thymi, the pattern of

autofluorescent cells was as in the differentially involuted thymi of normal rats of the same age. This indicated that the development of these cells is not dependent on a process starting with thymic involution. Secondly, rats aged 3 weeks were placed on a fat-free diet until they reached 10 weeks. Their considerably involuted thymi contained as many autofluorescent cells as thymi of normal rats of the same age. This revealed the endogenous origin of the lipids of their granules as well as the fact that the formation of these cells is independent of thymic lymphocytopoiesis and lymphopoiesis, both phenomena having been considerably reduced by the inanition. Finally, rats aged 2 months were cortisone-treated or the thymus was irradiated. Thymic autofluorescent cells remained at the cortico-medullary junction while, in the cortex, macrophages were actively engulfing degenerating lymphocytes. This demonstrated that the thymic autofluorescent cells are not mobilizable macrophages.

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The Separate Determination of Human Pepsin and Gastricsin.* (31231)

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The isolation and the study of 2 proteolytic enzymes, pepsin and gastricsin, from human gastric juice have been previously reported from our laboratory(1,2). Separate determination of the 2 enzymes from a mixture has been difficult because although the pH optima of the two enzymes are different(2), their respective proteolytic activities remain high throughout the acidic pH range. Thus quantitative analysis of pepsin and gastricsin separately required a fractionating technic. Such a method using a small ion-exchange column was developed(3). It allowed a quantitative measurement of the 2 enzymes in a solution with only about 5% error. The present communication describes a much less tedious method based on the discovery that the synthetic dipeptide substrate, N-acetyl-L-phenylalanyl-L-diiodotyrosine (APDT) is hydrolyzed by human pepsin but not by gastricsin. Baker previously reported that porcine pepsin also hydrolyzed this substrate(4). In the light of this finding, a method has been devised which allows a simple measurement of the 2 enzymes in a mixture. The principle of the method is to determine the amount of pepsin present using APDT as the substrate

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