

tine-induced abscesses with labeled antibody showing a slightly greater abscess localization than labeled fibrinogen in non-EACA treated rats. Treatment with EACA enhances abscess retention of both proteins, the effect being more pronounced in the case of labeled fibrinogen. When two abscesses are present in a single rat, radioactivity is distributed equally between the two abscesses. However, each of the two abscesses accumulates and retains less radioactivity than does an abscess present in a rat bearing only one such abscess.

In general, one may wonder how it is possible to have antifibrinogen antibody circulating in the presence of plasma fibrinogen. Over a wide range of antibody concentration, I^{131} -antibody preparations of the type used in our studies will rapidly form precipitates with rat fibrinogen if the two are mixed together at approximately equimolar concentrations; however, if the antigen is present in great excess, the antibody-antigen complex is soluble. The longer biological half-life in the blood of the I^{131} -antibody compared with that of fibrinogen has been noted by others (3). Presumably, it is due either to resistance of the complex to normal mechanisms of fibrinogen metabolism, to dissociation of the antibody from fibrinogen as fibrinogen is metabolized, or a combination of these factors.

Summary. An investigation of the effect of orally administered 5% ϵ -aminocaproic acid (EACA) in drinking water on the behavior of

intravenously injected I^{131} -fibrinogen and I^{131} -antibody to fibrinogen was carried out in both normal rats and rats with abscesses produced by subcutaneous injection of turpentine. Treatment with EACA had no demonstrable effect on either whole-body retention or blood half-life of either I^{131} -fibrinogen or I^{131} -antibody in normal rats. The most striking effect of EACA was to decrease greatly the rate of removal of I^{131} from the abscess area. Six days after injection of I^{131} -fibrinogen, 11% of the injected I^{131} was still present in single abscesses of EACA treated rats while in non-EACA treated rats the I^{131} retained was less than 1% of the injected dose. Similarly, 6 days after injection of I^{131} -antibody 20% of the injected I^{131} was present in abscesses of EACA treated animals and only 4% in abscesses of rats not receiving EACA. In animals with 2 abscesses, the amount of I^{131} deposited was greater but not double the amount deposited in single abscesses. This evidence supports the hypothesis that EACA is a potent inhibitor of fibrinolysis *in vivo*, as well as *in vitro*.

1. Bale, W. F., Spar, I. L., Goodland, R. L., Izzo, M. J., *Fed. Proc.*, 1961, v20, 58.

2. Mutschler, L. E., *Proc. Soc. Exp. Biol. and Med.*, 1964, v115, 1019.

3. Day, E. D., Planinsek, J. A., Pressman, D., *J. Nat. Cancer Inst.*, 1959, v23, 799.

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Effect of Thymectomy on Mast Cells in Mice.* (29108)

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The number of new mast cells produced in adult mice is very small according to radioautographic experiments with thymidine- H^3 (1). Proliferation of existing mast cells (2) could account for the few new mast cells that were identified by their radioactivity (3). There was no evidence of a precursor cell transforming into a mast cell (3). Recently,

Ginsberg and Sachs (4,5) reported inducing mast cell formation from thymus cells in tissue culture. They suggested that the thymus may be a normal source of mast cells. The radioautographic experiments on normal mast cell turnover were based on the premise that any proliferating cell population would be labeled by thymidine- H^3 during the course of the experiment. However, since small lymphocytes in the blood can circulate for a long time

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TABLE I. Number of Mast Cells in Dermis and Tongue of Mice Thymectomized at Birth and Unoperated Littermates.

Litter	Treatment	Wt (g)	Dermis—		Tongue—	
			Cells	Area (mm ²)	Cells	Area (mm ²)
1	thymectomized	7	142	1.68	95	5.38
	unoperated	7	152	1.41	76	5.38
2	thymectomized	7	297	2.19	91	7.75
	unoperated	7	208	2.34	73	7.16
3	thymectomized	9	168	1.49	92	9.15
	unoperated	9	157	2.50	71	6.96
total	thymectomized		607	5.36	278	22.28
(7-9 g)	unoperated		517	6.25	220	19.50
avg	thymectomized		113.2		12.5	
(per mm ²)	unoperated		82.7		11.3	
4	thymectomized	16	221	2.68	343	11.20
	unoperated	17	306	3.18	293	14.10
5	thymectomized	18	273	6.59	302	11.02
	unoperated	18	316	2.48	282	9.91
6	thymectomized	22	272	3.31	317	9.76
7	thymectomized	23	109	.94	249	9.56
total	thymectomized		875	13.52	1211	41.54
(16-23 g)	unoperated		622	5.66	575	24.01
avg	thymectomized		64.7		29.2	
(per mm ²)	unoperated		109.9		23.9	
total	thymectomized		1482	18.88	1489	63.82
(7-23 g)	unoperated		1139	11.91	795	43.51
avg	thymectomized		83.8		23.3	
(per mm ²)	unoperated		95.6		18.3	

and only a small percentage of them would be labeled in this type of experiment(6), transformation of small lymphocytes into mast cells could have gone undetected. To find out whether lymphocytes from the thymus transformed into mast cells in the post-natal period, the size of the mast cell population in skin and tongue of thymectomized mice was compared with that of their unoperated littermates.

Materials and methods. Newborn mice of the A/J strain were anesthetized by hypothermia. The thoracic cavity was opened by cutting through about 3 ribs alongside the sternum. The thymic lobes were removed with wire loop tweezers. The skin incision was closed and held in place with a coating of celloidin. Mice were killed at various time intervals and the thoracic cavity of the thymectomized mice was examined at autopsy to make sure no thymic tissue remained. Skin and tongue from 7 thymectomized and 5 control mice were fixed in Carnoy's solution, sectioned, and stained with toluidine blue, aldehyde fuchsin, or Bismark brown. Cell counts were made in the dermis of skin and throughout the tongue.

Results. A total of 2621 mast cells was

counted in dermis and 2284 in tongue. Since the frequency of mast cells as determined by the 3 different stains was similar, the counts are differentiated only by tissue and animal in Table I. In young (7 to 9 g) thymectomized mice, the average number of mast cells per mm² was 113.2 in dermis and 12.5 in tongue. In control mice, the averages were 82.7 for dermis and 11.3 for tongue. The concentration of mast cells per mm² in older mice (16 to 23 g) averaged 64.7 in dermis and 29.2 in tongue if thymectomized, 109.9 in dermis and 23.9 in tongue without thymectomy.

To test the significance of differences in mast cell frequencies between thymectomized and unoperated littermates the count from the larger area in each pair was reduced to be proportionate to the smaller area. In this sample of 15 paired variates from the 3 types of stain and 5 litters, the probability of differences in the mast cell counts being random was greater than 0.10 for dermis and for tongue. Some individual values differed considerably, but wide variations are common in mast cell counts(3).

Discussion. If the concentrations of mast cells in the areas of skin and tongue examined

were representative of the total mast cell population in the organism, then neonatal thymectomy did not significantly reduce the size of this cell population. These results are consistent with the proposal that mast cells constitute an independent, self reproducing population in the postnatal mouse. Since runtting disease was not seen in this series, peripheralization of thymocytes may have occurred before thymectomy. However, if the thymus is responsible for starting the mast cell population, rather than maintaining it, this would have to occur in the embryo, since mast cells are present at least as early as 15 days postconception in the mouse(7). That is, seeding of other tissues with lymphocytes by the thymus cannot have the same significance for mast cells as it does for immunological reactions by lymphoid cells. A difference of a day or two in the time of neonatal thymectomy could not influence the initiation of the mast cell population since the latter had already been initiated at least 4 or 5 days earlier than this. Therefore, while this experiment

does not provide information on the embryonic origin of mast cells, it does show that the mast cell population in postnatal mice is not dependent on the thymus as a source of new mast cells.

Summary. Neonatal thymectomy did not significantly reduce the size of the mast cell population in young and adult mice. Consequently, the thymus is not necessary for mast cell production during postnatal development.

1. Walker, B. E., *Nature*, 1961, v192, 980.
2. Allen, A. M., *Lab. Invest.*, 1962, v11, 188.
3. Walker, B. E., O'Steen, W. K., *PROC. SOC. EXP. BIOL. AND MED.*, 1963, v113, 183.
4. Ginsberg, H., *Ann. N. Y. Acad. Sci.*, 1963, v103, 20.
5. Ginsberg, H., Sachs, L., *J. Nat. Cancer Inst.*, 1963, v31, 1.
6. Bintliff, S., Walker, B. E., *Am. J. Anat.*, 1960, v106, 233.
7. Walker, B. E., *J. Embryol. Exp. Morph.*, 1961, v9, 22.

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Influence of Estrogen on Anterior Pituitary Lactogen Production *in vitro*.^{*} (29109)

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Reece and Turner(1) first reported that estrogen administration to rats increased pituitary lactogen content. A similar increase in pituitary lactogen after estrogen injection has been reported for guinea pigs(2,3) and rabbits(4). The addition of estrogen to anterior pituitaries cultured *in vitro* has been reported to increase oxygen consumption of rat(5) and human(6) adenohypophyses. Recently, Nicoll and Meites(7) observed an increase in lactogen production by rat pituitaries cultured *in vitro* when estrogen was added to the medium. It has also been noted that rat pituitaries estrogenized *in vivo* were capable of increased lactogen production *in*

vitro(8). The purpose of this endeavor was to examine the effects of estrogen on anterior pituitary lactogen production *in vitro* by adding estrogen to the medium, by estrogenizing pituitaries *in vivo* and subsequent *in vitro* culture, and by culturing both pituitaries and hypothalami together with estrogen added to the medium.

Materials and methods. Anterior pituitaries (AP) were obtained from female hooded Norway rats approximately 3 months of age (165-175 g body weight) and cultured *in vitro* according to the technique of Meites and co-workers(9). A total of from 6 to 12 AP were cultured per experiment, and each pituitary was divided into either 4 or 6 pieces. In the estradiol *in vitro* and the AP-hypothalamus co-culture studies, a piece from

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