

mones, University Press, Cambridge, 2nd Ed., 1949, p40.

11. Greep, R. O., *Sex and Internal Secretions*, Williams & Wilkins Co., Baltimore, Md., 3rd Ed., 1961, v1, p257.

12. Siperstein, E., Nichols, C. W., Jr., Griesbach, W. E., Chaikoff, I. L., *Anat. Rec.*, 1954, v118, 593.

13. Rowlands, I. W., Williams, P. C., *J. Endocrinol.*, 1943, v3, 310.

14. McCormack, C. E., Meyer, R. K., *Proc. Soc. Exp. Biol. and Med.*, 1962, v110, 343.

15. Lamond, D. R., *J. Endocrinol.*, 1958, v17, 218.

Received July 26, 1962. P.S.E.B.M., 1962, v111.

Effect of Adrenalectomy and Adreno-Demedullation on Experimental Edema as Measured under Repeated Ether Anesthetizations.* (27859)

ALAN C. LEVY, THOMAS H. BEAVER, ROBERT D. STRAIN AND DORSEY E. HOLTkamp

Scientific Laboratories, The Wm. S. Merrell Co., Division of Richardson-Merrell, Inc., Cincinnati, Ohio

Evidence has been reviewed which shows that various forms of stress cause an increase in adrenal activity with a subsequent release of corticosteroids and catecholamines(1). Since anesthesia with ethyl ether releases epinephrine(2), our experiments were performed to determine the effect of ether on experimental, mustard-induced, hind limb edema formation in adrenalectomized, adreno-demedullated and intact rats. The studies were designed to shed light on the role played by the medullary and cortical portions of the adrenal gland in the general process of inflammatory edema.

Method. Male Sprague-Dawley rats (Charles River Breeding Laboratories-CD), 150-220 g, were used in all studies. Adrenalectomies were performed bilaterally the day prior to the experiment and the rats were allowed 0.9% saline *ad libitum* thereafter. Adrenal demedullations were done bilaterally about 30 days before the animals were used (3). Ethyl ether (U.S.P.) was used for the surgery and all limb volume determinations conducted on anesthetized animals.

Limb volumes were determined by volumetric displacement according to a previously described method(4,5) adapted for our use. The foot, up to the hair line, was placed in a tube of mercury and the volume, in milliliters, determined by the amount of mercury displaced. The edema was induced by the sub-

TABLE I. Schedule of Limb Volume Determinations.

No. of limb volume determinations after initial determination	Time of determinations (hr after mustard inj)
1	6
2	2, 6
3	1, 2, 6
4	1, 2, 4, 6
5	.5, 1, 2, 4, 6

plantar injection of 0.1 ml of an irritating 2% aqueous mustard[†] suspension(6) into the left hind limb. The injections were given immediately after the initial limb volume reading. The amount of edema present was calculated by subtracting the initial limb volume of each animal from the subsequent volumes recorded at different time intervals after injection of the irritant. Limb volumes were determined before and from 1 to 5 times after the mustard injection. The time schedule shown in Table I was followed for intact, adrenalectomized and adreno-demedullated animals given ether for each determination as well as for intact rats in which limb volumes were determined without anesthesia. All presented data are the result of 3 experiments done on different days with 5 to 8 rats per group.

Results. Table II shows the amount of

* We thank Mrs. Christina Lockwood for assisting in limb volume determinations.

[†] Colman's Mustard (double superfine) distributed by R. T. French Co., Rochester, N. Y. Mustard used in these experiments was from a single lot well mixed to assure uniformity of irritancy.

TABLE II. Influence of Number of Prior Limb Volume Determinations on Mean 6-Hour Limb Volume of Rats Injected Sub-Plantarily with Mustard at 0-Time.

Group	No. edema determinations after irritant administration	No. rats	Edema (ml)†
Ether-Intact	5	18	.32 ± .026
	4	18	.36 ± .026
	3	18	.32 ± .031
	2	18	.33 ± .027
	1	18	.49 ± .030
	Mean*	72*	.33 ± .013*
Ether-Adrex	5	16	.46 ± .032
	4	16	.42 ± .033
	3	18	.47 ± .027
	2	18	.47 ± .036
	1	18	.45 ± .020
	Mean	86	.45 ± .012
Ether-Demed.	5	18	.42 ± .028
	4	18	.40 ± .034
	3	18	.43 ± .023
	2	18	.43 ± .037
	1	18	.45 ± .029
	Mean	90	.43 ± .013
No ether-Intact	5	24	.49 ± .036
	4	24	.50 ± .042
	3	24	.49 ± .025
	2	24	.50 ± .032
	1	24	.43 ± .034
	Mean	120	.48 ± .015

* Mean of ether-intact excludes data from single 6-hr determination.

† Mean ± standard error.

edema (mean ± S. E.) present in the hind limbs of various groups of rats at 6 hours after the mustard injection. *Intact ether.* Two to 5 volume determinations after the mustard injection and under ether anesthesia, resulted, in intact rats, in about the same amount of edema (0.32 to 0.36 ml) at 6 hours. Intact rats subjected to a single 6-hour post-mustard determination under anesthesia had significantly ($P < 0.001$ -(7)) more edema (0.49 ml). *Adrenalectomized-ether.* In adrenalectomized rats, the degree of edema observable at 6 hours was not influenced by the number of prior anesthetizations. The amount of edema (0.42 to 0.47 ml) in these rats was of about the same magnitude as that seen (0.49 ml) in intact rats after the single post-mustard 6-hour determination. *Adreno-demedullated-ether.* The edema in adreno-demedullated animals ranged from 0.40 to 0.45 ml. These volumes were slightly, but

insignificantly, lower than those observed in adrenalectomized rats (0.42 to 0.47 ml). All volumes were within the same range regardless of the number of times the rats were anesthetized. The amount of edema (0.40 to 0.45 ml) was greater than that (0.32 to 0.36 ml) observed in intact rats that were anesthetized more than once after the mustard injection. *Intact-no ether.* Limb volumes in the non-anesthetized intact animals subjected to 2 or more determinations showed edema (0.49 to 0.50 ml) of approximately the same amount as that (0.49 ml) noted in intact animals subjected to a single post-mustard anesthetization. Although the non-anesthetized intact group which had only a single post-mustard 6-hour volume determination, appeared to have less edema (0.43 ml) than the other groups (0.49 to 0.50 ml), the difference had a statistical probability of only $0.2 > P > 0.1$.

Averaging all of the 6-hour limb volumes (Table II) within each of the 4 experimental groups (except for the single 6-hour determination in anesthetized intact rats) showed that the intact rats subjected to 2 or more post-mustard determinations under anesthesia had significantly less edema than did either the anesthetized adrenalectomized, anesthetized demedullated or the non-anesthetized intact rats ($P < 0.001$). A time-sequence curve in which results (except for the single 6-hour determination in anesthetized intact rats) were combined from many experiments showed that the degree of edema decreased with time in all 4 groups, but that it diminished most rapidly (Fig. 1) in anesthetized intact rats.

Discussion. Two or more anesthetizations with ether within a 6-hour period decreased the amount of experimental edema inducible in the hind limb of intact rats. This decrease was absent in comparably anesthetized rats which had been either bilaterally adrenalectomized or bilaterally adreno-demedullated. Also, non-anesthetized intact animals did not have this decrease. Adrenal corticoids, adrenal catecholamines and pituitary ACTH (via adrenal cortical stimulation) have a profound influence on the edematous aspect of the inflammatory process(8). Since stress is known to cause an increased release of these

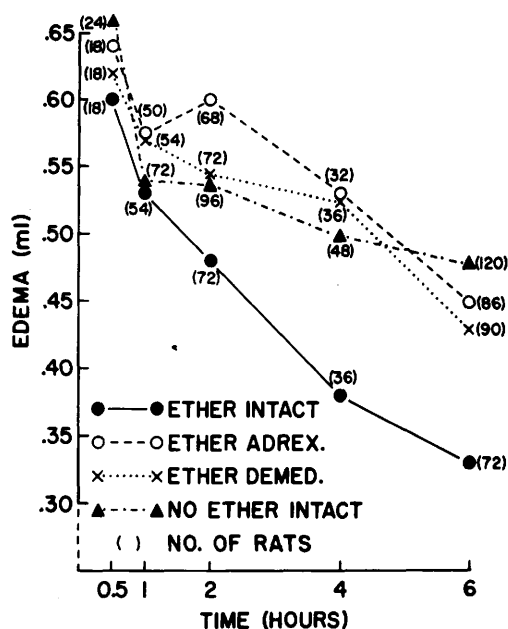


FIG. 1. Composite of mean hind limb volumes of anesthetized and non-anesthetized rats for the 6-hr period after subplantar injection of mustard except that data from the single 6-hr determination in anesthetized intact rats were not included.

hormones(1,9,10,11) the decrease in edema observed with the 2 or more anesthetizations may very likely be due to adrenal stimulation. Furthermore, it appears that the effect of the ether was mediated *via* the adrenal medulla rather than the cortex and that hormones of the adrenal medulla are of importance in control of inflammatory edema, or alternatively, that the process of demedullation destroyed a sufficient portion of the adrenal cortex to reduce corticoid output with consequent reduction of edema inhibition. The latter is less likely. Kelemen(8) has concluded that hormones of the adrenal medulla yield "acute inhibition" of edema but that no adreno-cortical substance produced this acute inhibition. In our experiment, the 6-hour lapse between the 0-hour determination and the 6-hour de-

termination in anesthetized rats appeared to be sufficiently long for the acute anti-edema effect to have subsided.

Summary. In experimental anti-edema studies, rats were subjected to varying numbers of hind limb volume measurements during a 6-hour period. Edema was induced by sub-plantar injection of mustard. Intact rats having 2 to 5 post-mustard determinations while under light ether anesthesia, had less edema at 6 hours than those having only a single 6-hour measurement. This difference was not observed in similarly anesthetized bilaterally adrenalectomized or bilaterally adreno-demedullated rats, or in unanesthetized intact rats. These results suggest that the adrenal medulla is implicated in the control of experimental inflammatory edema.

1. Ramey, E. R., Goldstein, M. S., *Physiol. Rev.*, 1957, v37, 155.
2. Goodman, L. S., Gilman, A., *The Pharmacological Basis of Therapeutics*, 2nd ed, The Macmillan Co., N. Y., 1955, p61.
3. Ingle, D. J., Griffith, J. Q., in *The Rat in Laboratory Investigation*, ed., E. J. Farris and J. Q. Griffith, J. B. Lippincott, Philadelphia, 1949, p444.
4. Wilhelmi, G., Domenjoz, R., *Arzneimittel-Forsch*, 1951, v1, 151.
5. Stucki, J. C., Thompson, C. R., *Am. J. Physiol.*, 1958, v193, 275.
6. Coutu, L. L., *Presse Med.*, 1950, v58, 781.
7. Snedecor, G. W., *Statistical Methods*, 4th ed., Iowa State College Press, 1937, p77.
8. Kelemen, E., *Permeability in Acute Experimental Inflammatory Oedema*, Publishing House, Hungarian Acad. of Sci., Budapest, 1960.
9. Wells, H., Briggs, F. N., Munson, P. L., *Endocrinology*, 1956, v59, 571.
10. Mahfouz, M., Ezz, E. A., *J. Pharmacol. and Exp. Therap.*, 1958, v123, 39.
11. Price, H. L., Linde, H. W., Jones, R. E., Black, G. W., Price, M. L., *Anesthesiology*, 1959, v20, 563.

Received July 26, 1962. P.S.E.B.M., 1962, v111.