OUTCOME OF SUBCUTANEOUS INFILTRATION OF LEVOPHED-REGITINE SOLUTIONS IN 36 RABBITS

LEVOPHED, mg./L.	GROUP: A	B	C	D	E	F
32	++	++	++	oo	oo	oo
16	++	++	+o	oo	oo	oo
8	++	oo	oo	oo	oo	oo

0 0.5 1.0 2.5 5.0 10.0

REGITINE, mg./L.

+ NECROSIS o NO NECROSIS

FIG. 1. Effect of varying concentrations of phentolamine in prevention of levarterenol necrosis.

the previously reported observations that ischemic necrosis uniformly follows slow subcutaneous infiltration of 250 cc of levarterenol solution into the rabbit abdomen. McGinn and Schluger(4) used a solution equivalent to 16 mg of levarterenol/liter. Similar changes were produced in our study with solutions containing 8, 16, and 32 mg levarterenol. Size of sloughs varied directly with concentration of levarterenol.

Phentolamine given simultaneously with levarterenol has a potent inhibitory effect on cutaneous vasoconstriction of the latter (Fig. 1). As little as 0.5 mg counteracted the effect of 8 mg of levarterenol. A concentration of 1 mg phentolamine diminished the vasoconstriction produced by 16 and 32 mg levarterenol judging by the small size of resultant slough. The lowest phentolamine dose to abolish the ischemic effect of all 3 strengths of levarterenol was 2.5 mg. The same beneficial response was obtained with 5 and 10 mg phentolamine.

These experiments were carried out to determine smallest amount of phentolamine that will abolish the local vasoconstrictor action of doses of levarterenol usually administered to patients in shock. If results in normal rabbits can be extrapolated to patients in shock, then mixtures containing 2.5 mg phentolamine might be free from the ischemic hazard. However, circulation in extremities of patients in shock is not comparable to that in the skin of abdomen of normal rabbits. Intense vasoconstriction commonly associated with shock may increase the requirement for phentolamine.

Summary. 1. Subcutaneous infiltration of 250 cc of solutions containing 8, 16, or 32 mg levarterenol/liter for 2 hours regularly produced slough of skin on abdomen of rabbits. 2. All levarterenol-phentolamine mixtures that contained 2.5, 5, and 10 mg phentolamine did not produce slough. Those containing 0.5 and 1 mg phentolamine prevented slough only for mixtures with 8 mg levarterenol. Sloughs were reduced with mixtures containing 16 and 32 mg levarterenol and 1 mg phentolamine.

1. Zucker, G., *J.A.M.A.*, 1957, v163, 1477.

2. McGinn, J. T., Schluger, J., Di Gregoria, N. J., *N. Y. State J. Med.*, 1956, v56, 1950.

3. Zucker, G., Levine, J., *A.M.A. Arch. Int. Med.*, 1959, v104, 607.

4. McGinn, J. T., Schluger, J., *Am. J. Surg.*, 1956, v92, 594.

Received October 2, 1959. P.S.E.B.M., 1960, v103.

Serotonin Studies on Mouse Tissues.* (25481)

ROBERT M. DONALDSON, JR.,† SAUL MALKIEL AND SEYMOUR J. GRAY

Depts. of Medicine and Pathology, Harvard Medical School and Peter Bent Brigham Hospital, Boston

Anaphylaxis in the mouse apparently differs from that observed for other species with respect to difficulty of sensitization and shock organs and mediators concerned. Since histamine is believed to be of relatively little importance in this reaction(1), attention has

* Supported by grant from N.I.H., U.S.P.H.S.

† Present address: Clinical Investigator, V. A. Hospital, Boston.

TABLE I. Serotonin Content of Mouse Tissues.

Tissue	Serotonin values		
	Normal	Tumor*	Tumor-anaphylactic shock
Kidney	1.3 ± .1 (4)†	39.8 ± 13.7 (4)	9.9 ± 12.6 (5)
Lungs	2.7 ± .8 (2)	27.1 ± 12.2 (3)	3.5 ± 1.3 (6)
Small intestine	3.1 ± .3 (11)	12.9 ± 6.4 (6)	1.1 ± .9 (5)
Liver	0 ±0 (8)	81.6 ± 33.2 (10)	20.9 ± 5.6 (7)
Spleen	5.9 ±2.0 (3)	213 ± 40.4 (5)	175 ± 37.3 (7)
Tumor		169 ± 36.2 (6)	192 ± 41.8 (7)
Ascitic fluid		44.1 ± 80.1 (2)	

* Bearing actively growing, palpable mastocytoma.
† $\gamma/g \pm$ S.D. No. in parentheses = No. of determinations.

been focused on serotonin(2-5). The latter is of particular interest in this species because of abundance and widespread presence of large numbers of mast cells in the loose connective tissue of skin, snout, paws, etc. and along lymphatic channels, small blood vessels and nerves of forestomach, tongue, lungs, mesentery, intestines, etc.(6). The mast cell is probably responsible for secretion and liberation of serotonin(7). There is no evidence (4) that serotonin is released from tissues other than platelets in the anaphylactic reaction. A study of anaphylaxis in presence of a transplantable mouse mastocytoma, consisting essentially of mast cells and potentially capable of secreting large quantities of serotonin, indicated that neither presence of tumor nor its secretory products had any effects on the reaction(8). Serotonin content of tissues of tumor bearing mice was determined and the results compared to those obtained from normal mice. Alteration in serotonin content was subsequently determined in these mice following anaphylactic reaction.

Methods. The mice used were of LAF_1 strain treated as described previously(8). For purposes of serotonin determinations, the animals were divided into 3 groups. Group I consisted of control animals. Groups II and III consisted of mice bearing transplantable mast cell tumors. The animals in Group III were those which were sensitized and in which anaphylactic shock was elicited. Tissues were frozen immediately in a dry ice-cellosolve bath after animals were sacrificed or died in anaphylactic shock. The 2 kidneys, liver, small intestine and tumor from individual mice each provided sufficient tissue for serotonin analysis. It was necessary, however, to pool the lungs and spleens from several animals to measure tissue serotonin levels. Serotonin was assayed by the spectrophotofluorimetric method of Bogdanski *et al.*(9).

Results. Tissue serotonin levels of above 3 groups are presented in Table I. Tissue content of serotonin in mice bearing the transplantable mastocytomas was markedly increased, and most increased in liver and spleen. A high serotonin content of mast cell tumors was also found. Ascitic fluid from 2 mice with mastocytomas contained significant quantities of serotonin.

When tissue serotonin of tumor animals was compared to that observed in tumor mice subjected to anaphylactic shock, a significant decrease, ($P<.01$), in serotonin concentration was noted in kidneys, lungs, small intestine and liver. Serotonin content of small intestine and lung was reduced to values approaching those observed in control animals. Following anaphylaxis a decrease in serotonin content was not found in spleen or mast cell tumor.

Discussion. Although significant amounts of serotonin have been found in transplantable mastocytomas of mice(10), information concerning serotonin levels of various tissues of mice bearing this tumor has not been previously available. Our data suggest that a marked increase in serotonin content may be observed in tissues of such animals. The highest serotonin levels were found in spleen, where the amine is probably not being metabolized but is apparently being stored in platelets.

Previous studies showing a relatively high concentration in the lung have implicated serotonin in the anaphylactic reaction in the mouse(3). It is of interest that significant decreases in tissue serotonin content were ob-

served during anaphylaxis in the mast cell tumor mice. Kidneys, lungs, small intestine and liver are all thought to be capable of metabolizing free serotonin(11), and, in these organs, anaphylaxis was associated with a striking decrease in the elevated serotonin levels. A similar reduction was not noted in spleen or in mast cell tumors. Presumably in these tissues serotonin was either not released or, if released from cells, was not subsequently metabolized. Reduction in serotonin content of whole blood is accompanied by a fall in number of platelets during anaphylaxis in the rabbit(4). However, no evidence of release from tissue has been obtained(5). In tissues of mice bearing mastocytomas the decreases in serotonin concentration were striking.

The high serotonin content observed in tissues of mice with mast cell tumors suggest that such experimental animals might prove useful in the study of effects of serotonin in a situation where the amine is chronically present in large amounts. The mechanisms by which serotonin is bound to or released from tissues remain poorly understood. In view of the striking changes observed in tissue serotonin content, further studies utilizing organs of mice with mast cell tumors may help clarify these mechanisms.

Summary. Tissues of mice bearing mastocytomas are rich in serotonin. A significant decrease in serotonin content occurred in kidneys, lungs, small intestine and liver of these mice following anaphylactic shock.

1. Malkiel, S., Hargis, B. J., *J. Allergy,* 1952, v23, 352.

2. Fink, M. A., Rothlauf, M. V., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 447.

3. Weissbach, H., Wallkes, T. P., Udenfriend, S., *Science,* 1957, v125, 235.

4. Waalkes, T. P., Weissbach, H., Bozicevich, J., Udenfriend, S., *J. Clin. Invest.,* 1957, v36, 1115.

5. ———, PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 479.

6. Weiser, R. S., *J. Allergy,* 1957, v28, 475.

7. Benditt, E. P., Rowley, D., *J. Exp. Med.,* 1956, v103, 399.

8. Malkiel, S., Hargis, B. J., PROC. SOC. EXP. BIOL. AND MED., in press.

9. Bodganski, D. F., Pletscher, A., Brodie, B. B., Udenfriend, S., *J. Pharm. Exp. Therap.,* 1956, v117, 82.

10. Sjoerdsma, A., Waalkes, T. P., Weissbach, H., *Science,* 1957, v125, 1202.

11. Udenfriend, S., Titus, E., Weissbach, H., Peterson, R. E., *J. Biol. Chem.,* 1956, v219, 335

Received October 21, 1959. P.S.E.B.M., 1960, v103.

Absorption of Cholesterol-4-C^{14} Oleate* (25482)

LEON SWELL, HENRY FIELD, JR., AND C. R. TREADWELL

Vet. Admin. Center, Martinsburg, W. Va., and Dept. Biochemistry, School of Medicine, George Washington University, Washington, D.C.

Studies(1,2) using blood cholesterol levels as an indicator of cholesterol absorption in chicks and rats have demonstrated that feeding of free cholesterol leads to a higher blood cholesterol level than feeding of the long chain fatty acid esters of cholesterol. Vahouny and Treadwell(3) examined lymph after feeding of different cholesterol esters and found that free cholsterol was absorbed more rapidly than the long chain fatty acid esters of cholesterol. They suggested that only free cholesterol was transferred from the lumen into the mucosa. Pihl(4) reported that fed cholesterol esters were extensively hydrolyzed in the intestine and were not absorbed to a greater extent than free cholesterol. Favarger and Metzger(5) fed D-cholesterol oleate and demonstrated hydrolysis of this labeled ester in the lumen of intestine. They also found that in the intestinal wall of such animals the labeled cholesterol was present principally in the free form. When free D-cholesterol was

* This investigation was aided by research grants from U.S.P.H.S. and Am. Heart Assn.