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Local Resistance to a Lethal Dose of Formalin.*

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According to Selye[1,2,3] an animal exhibiting an "alarm reaction" in response to a given chemical is able to resist a lethal dose of a chemical of different nature because of a non-specific mechanism of general defense initiated by this "alarm reaction" and called "reaction of adaptation." In repeating certain of Selye's experiments, preliminary to studies on induced immunity to transplantable leukemia, evidence was obtained that questions the general distribution of the cross resistance between the two chemicals employed.

The present communication is concerned with a study of the location of resistance to formalin and adrenalin induced by formol treatment when the sites of pretreatment and lethal doses were varied.

The experimental material consisted of male and female mice of strain C58,[4,5] whose ages varied between 5 and 7 weeks and whose weights varied between 13 and 22 g. These animals received the standard food and care of this laboratory.[6] Solutions of formalin of 4% and 10% (prepared from the commercial solution) and adrenalin in aqueous solution of 1:1000 were used as "alarming" stimuli in subcutaneous injections. The minimum lethal doses of these substances were found to vary according to the site of injection (Table I).

Treatment previous to the lethal dose was administered within a period of 48 hours, using 4 injections. The doses used when all 4 injections were given at the same site were the following: 0.10, 0.20, 0.30, and 0.40 cc of 4% formalin; 0.05, 0.10, 0.12, and 0.15 cc of 10% formalin; or 0.06, 0.08, 0.10, and 0.10 cc of adrenalin; in different experiments the site was the skin of the abdomen, the dorsolumbar region, or the ventral surface of the left hind leg. When the injections were made at different sites in the same mice, they received either 4 doses of 0.15 or 0.20 cc of 4% formalin, or 4 injections of 0.06 cc of adrenalin, the sites being: the extremities, and the abdominal, anterior thoracic, dorsolumbar, and dorsocervical regions. In a few experiments each dose of the treatment with progressive amounts of the chemicals was divided into 4 subdoses, and

TABLE I.

Minimum Lethal Dose of Formalin and Adrenalin Given Subcutaneously in C58 Mice According to the Sites.

Male weight: 18 to 22 g; female weight: 13 to 20 g.

Chemical and concentration		Dorsolumbar	Abdominal	Anterior thoracic	Medial surface hind left leg	Dorsocervical
Formalin	10%	0.20	0.20	0.20	1.15	0.10
Formalin	4%	0.50	0.50	—	—	—
Adrenalin	1%	0.15	0.12	—	—	—

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TABLE II.

Number of Mice Surviving Lethal Dose of Formalin or Adrenalin When Injected in the Site of Pretreatment or in a Different Site.

	Pretreatments			Lethal dose		No. of mice	No. surviving after					Total surv.
	Chemical	Dosage	Sites	Chemical	Sites		1st injec.	2nd injec.	3rd injec.	4th injec.	L.D.	
1	Form.	incr.	same	—	—	34	34	34	33	33	—	33/34
2	,,	,,	diff.	—	—	28	28	18	11	3	—	3/28
3	,,	,,	same	Form.	same	88	87	85	84	74	65	65/74
4	,,	,,	,,	,,	diff.	96*	89	82	77	61	7	7/61
5	,,	equal	,,	,,	same	31	28	28	26	26†	13	13/22
6	,,	,,	diff.	,,	diff.	54	37	35	35	35	9	9/35
7	Adren.	incr.	same	Adren.	,,	16	16	15	13	11‡	5	5/ 5
8	Form.	,,	,,	,,	same	55	45	45	41	39§	36	36/38
9	,,	,,	,,	,,	diff.	26	26	26	26	26	3	3/26
10	Adren.	,,	,,	Form.	same	16	16	12	8	6	0	0/ 6
11	,,	equal	diff.	,,	diff.	10	9	7	4	3	0	0/ 3
12	Untreated controls			—	—	128					11	11/128
13	,,			Adren.	—	35					2	2/35

* One group of 21 animals received the treatment in the same site but each dose was distributed in 4 different sites.
†, ‡, § Only 22, 5, and 38 animals, respectively, were injected with the lethal dose.

administered at 4 different sites. The animals were usually subjected to the lethal test dose (the size of the dose varying according to the site) 12 hours after the last of the pretreatments, although in a few cases the lethal dose was administered 2, 3 or 6 days later. When death occurred, it was, in most experiments, at approximately the same time as the death of the controls without pretreatment.

Resistance to the preliminary treatment and lethal dose of formalin depends upon the site of administration (Table II). When progressive doses of this substance were injected into one site, 33 out of 34 animals resisted the 4th pretreatment (line 1), while there were only 3 survivals out of 28 cases when the 4 treatments were given in different sites (line 2). Resistance to the lethal dose also depends on the same factor. When it was administered at the site of pretreatments, 65 out of 74 mice survived (line 3); when the lethal dose was injected at a different site, only 7 out of 61 survived (line 4), a result similar to the controls without pretreatment (survival, 11 out of 128, line 12).

The pretreatment was less effective when sublethal doses of equal amount were used. Animals treated in this manner showed resistance to lethal dose injected at the same site in 13 cases out of 22 (line 5), and the animals which died did so after a longer interval than the controls which had been pretreated at different sites (survival, 9 out of 35, line 6).

Many of the animals treated with adrenalin died during the course of treatment, whether this substance was administered at the same site or at different ones. Some of them, subjected to a lethal dose after 4 preliminary injections, resisted adrenalin even when it was applied at a site different from that of the pre-treatment (survival, 5 out of 5, line 7).

In experiments with cross-resistance, 36 out of 38 mice pretreated with formalin resisted a lethal dose of adrenalin when all injections were at the same site (line 8); when the lethal dose was injected at another site, only 3 out of 26 survived (line 9), with the result that the survival rate approached that

TABLE III.
Anatomical Findings Soon After Treatment with 10% Formalin.
Group I—4 injections of 0.05, 0.10, 0.12, and 0.15 cc.
Group II—4 injections of 0.10, 0.15, 0.20 and 0.25 cc.

Experimental groups	No. of mice	Organ wt (mg)					Local lesion site inj.	General hyperemia
		Liver	Spleen	Thymus	Adrenal	Pancreas		
I	5	726 (600-800)	37 (30-38)	14 (12-18)	5.0* (4.4-5.5)	85 (68-99)	+++	++
II	5	814 (700-950)	46 (38-68)	21 (10-35)	4.5* (4.0-5.2)	95 (60-155)	+++	++
Normal (controls)	5	802 (720-880)	81 (64-127)	47 (41-60)	4.1 (3.8-4.5)	141 (90-179)	——	——

* Most of the animals showed adrenal changes: cortex pink and slightly transparent; pale medulla, serous membrane edema doubtful in Groups I and II after treatment. One spontaneous death in Group I and 3 in Group II.

of untreated controls injected with the same dose (2 out of 35, line 13). The animals treated with adrenalin did not show cross-resistance to formalin, whether the lethal dose was injected at the same or at a different site (lines 10 and 11).

Autopsy was made on various animals in order to investigate the anatomical symptoms of the alarm reaction and the reaction of the skin at the site of treatment (Table III). At the termination of the treatment, involution of the thymus was evident. Changes in the suprarenal and pancreas were less conspicuous, with a few exceptions. General hyperemia was present, and digestive hemorrhages were frequently observed in animals treated with adrenalin.

The skin showed changes only when formalin was used, and edema and congestion were apparent during the treatment; several days after the last injection, formation of a necrotic scar became evident.

Histological examination of animals treated with formalin, kindly made by Dr. Richard Miller, revealed the changes of the thymus mentioned under the description of the alarm reaction, increase of mitosis of the cortical cells of the suprarenal, and a necrotic and inflammatory type of skin alteration.

It appears that the induction of resistance to a lethal dose depends upon the nature of the chemical used and upon the site. The animals pretreated with adrenalin evinced resistance to the lethal dose of adrenalin of general character, at least in the one small experiment, but showed no cross-resistance to formalin, either local or general. On the contrary, the animals pretreated with formalin showed only a local resistance, whether they were subjected to a lethal dose of formalin or one of adrenalin. Although no general resistance was observed, the formalin-treatment produced the principal anatomical signs of Selye's "alarm reaction."

These differences observed in mice treated with formalin and adrenalin appear to have their explanation in the differing capacities of these substances to induce local lesions. While the first caused intense manifestations at the site of the injection, culminating in

the formation of a necrotic lesion of the skin, the second, on the other hand, did not produce macroscopically visible local lesions.

Nothing definitive can be said about the possible mechanism of the local resistance observed. It is quite possible that the inflammatory state provoked by the formalin diminished the absorption of the lethal dose.[7,8,9]

Although local resistance is not frequently encountered, it is not a new phenomenon. Rosenthal, Tabor, and Lillie observed that mice surviving tourniquet shock because of salt treatment would survive a repetition of the same shock without salt, if given in the same leg, but would die if it was given in a different leg.[10] Similarly, according to a review by J. Levy, animals in which resistance to arsenic has been built up *per os* do not tolerate a toxic dose administered subcutaneously.[11]

Summary. When mice were pretreated with adrenalin they showed general resistance to a lethal dose of this substance but not cross resistance to formalin, either local or general. On the contrary, animals pretreated with formalin revealed only a local resistance, whether they were subjected to a lethal dose of formalin or of adrenalin. These observations seem to question Selye's interpretation of some of his experiments on general adaptation to the same or different "alarming" stimuli.

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Renal Reabsorption of Methionine in Normal Dogs.*

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It is known that at normal plasma concentrations, reabsorption of amino acids from the glomerular filtrate is practically complete. Experiments in which the plasma concentration has been raised above normal levels have shown, however, that there are differences in the efficiency with which individual amino acids are reabsorbed. These differences are manifested in the rates of reabsorption of the various acids prior to the attainment of the maximal rate (Tm), and also in the levels at which the Tm is reached.[1-5]

The experiments to be described were designed to study the renal reabsorption of methionine. Shortly after they were concluded, experiments utilizing the microbiological method of analysis were reported[5] which indicated that the reabsorption of methionine is practically complete at plasma levels up to 115 mg % (equivalent to about 10.8 mg

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