NEUTRALIZATION OF HISTAMINE AND BURN TOXIN

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IN 1929, when the writer was associated with the Children's Surgical Division of the Cook County Hospital, he was impressed with the fact that in burns of moderate intensity in which healing was prolonged and especially when the inevitable superficial infection set in, there began the low grade fever, wasting, clammy skin and pallor usually associated with a low grade toxemia. Dressing and débriding these wounds daily, one could not divorce oneself from the idea that local absorption of some of the dead tissue must take place, and that it may account for at least some of the local edema and toxicity. Reasoning further, he rationalized that if the absorbed dead tissue acted as a toxin, then perhaps an antitoxin was produced. To this end blood was actually drawn from an old burn case who was awaiting skin grafts, but the opportunity to employ this serum did not present itself.

Later, while working in the Pathology Department of the same institution, postmortem observations in cases of burns revealed subcutaneous edema and internal dehydration, hyperemia and parenchymatous degeneration of the organs. In addition, and not infrequently, the occurrence of acute gastric ulcers presented themselves, from which bleeding had resulted in the exitus. At times the extent and severity of the burn was not marked.

As has already been reported (Rosenthal[11]), there is present in the blood of burned animals and humans a substance which causes contraction of the virgin guinea-pig uterus and is toxic to guinea-pigs. This substance was thought to be formed locally at the site of the burn, and only when present in large quantities could its presence be detected in the blood. A similar theory has been offered to explain anaphylactic shock (Dale and Laidlaw,[7] Biedl and Kraus,[4] Lewis and Grant,[8] Simonds and Brandes[12]). The following experiments were undertaken to determine if histamine and neutralizing substances were formed in burned animals and humans.

Method.—Blood was withdrawn periodically from the tail of second degree burned shoats and adult pigs under ether anesthesia, to determine the presence of neutralizing properties. The observations were made on the virgin guinea-pig uterus. To the serum, as well as to other fractions of the blood, 0.5 per cent phenol or chloroform (three drops to 20 cc.) was added immediately after withdrawal to prevent bacterial activity. All specimens were kept in an ice-box (2° C. to 4° C.) until used.

Similar observations were recorded for human blood, using serum from old burn cases where the lesions were healed or practically so.

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TABLE I

THE ACTION OF VARIOUS SERUMS ON HISTAMINE

Source	Type of Material	Time after Burn	Presence of Histaminoid Substances	Place and Time of Mixture			
				Room Temperature (30° C.–34° C.)	Incubation (37° C.)	Ice-Box (3° C.)	Combinations
Pig.....	Nor. S. (h)	—	0		++ (10 min.) 0 (3 hrs.)		
Pig.....	Nor. S.	—	0	++ (10 min.) ++ (30 min.) tr. (24 hrs.)	++ (10 min.) ++ (5¼ hrs.) + (6¼ hrs.) tr. (7 hrs.)	++++ (24 hrs.)	
Pig.....	Nor. cit. b. (h)	—	0		+++ (5 hrs.)		
Pig.....	Nor. cit. b.	—	0		+++ (5¼ hrs.)		
Pig.....	S.	2 mos.	+++		+++ (10 min.) +++ (25 min.)		
Pig.....	S. (h)	2 mos.	0 (h)		+++ (10 min.)		-
Shoat.....	S.	20 da.	0		+++ (10 min.) + (6 hrs.)	+++ (24 hrs.)	(24 hrs. 3° C.+ +++ 3 hrs. 30° C.+ 20 min. 37° C.)
Shoat.....	S.	28 da.	0		tr. (5¾ hrs.)		
Pig.....	S.	2 mos.	0	++ (35 min.) ++ (50 min.) + (75 min.) 0 (24 hrs.)	tr. (5½ hrs.) + (6½ hrs.) + (6¾ hrs.)	tr. (24 hrs.)	
Human.....	S.	13 mos.	tr.		++ (10 min.) ++ (5 hrs.)		
Human.....	S.	22 da.	tr.		++ (10 min.) ++ (5 hrs.)		
Human.....	S.	25 da.	tr.				
Histamine....	1 :100,000 dilution	—	++	++ (1 hr.) ++ (5 hrs.) ++ (24 hrs.)	++ (10 min.) ++ (4¾ hrs.)	++ (24 hrs.)	
Histamine (h).	1 :100,000 dilution	—	++		++ (10 min.)		
Ringer-Locke's	Phys. salt	—	0	++ (1 hr.) ++ (5 hrs.) ++ (24 hrs.)	++ (10 min.) ++ (3 hrs.) ++ (4¾ hrs.)	++ (24 hrs.)	(24 hrs. 3° C.+ ++++ 2 hrs. 30° C.+ 20 min. 37° C.)

++ = depression caused by 1 cc. of a 1 :100,000 dilution of histamine; S. = serum; nor. = normal; (h) = heated 60° C. for one-half hour; tr. = trace; cit. b. = citrated blood.

Results.—I. Counteracting Histamine.—Table I gives the results pertaining to the action of blood and serum upon histamine. All mixtures were made in equal proportions, using a 1:100,000 dilution of histamine in ½ to 1 cc. amounts. A two plus (++) reaction is to be interpreted as a contraction of the same degree as histamine. All other observations are recorded according to this standard. The solutions to be tested were all introduced into 35 cc. of Ringer-Locke solution in which the virgin ovary-uterus preparation was suspended (method described in previous publication[11]).

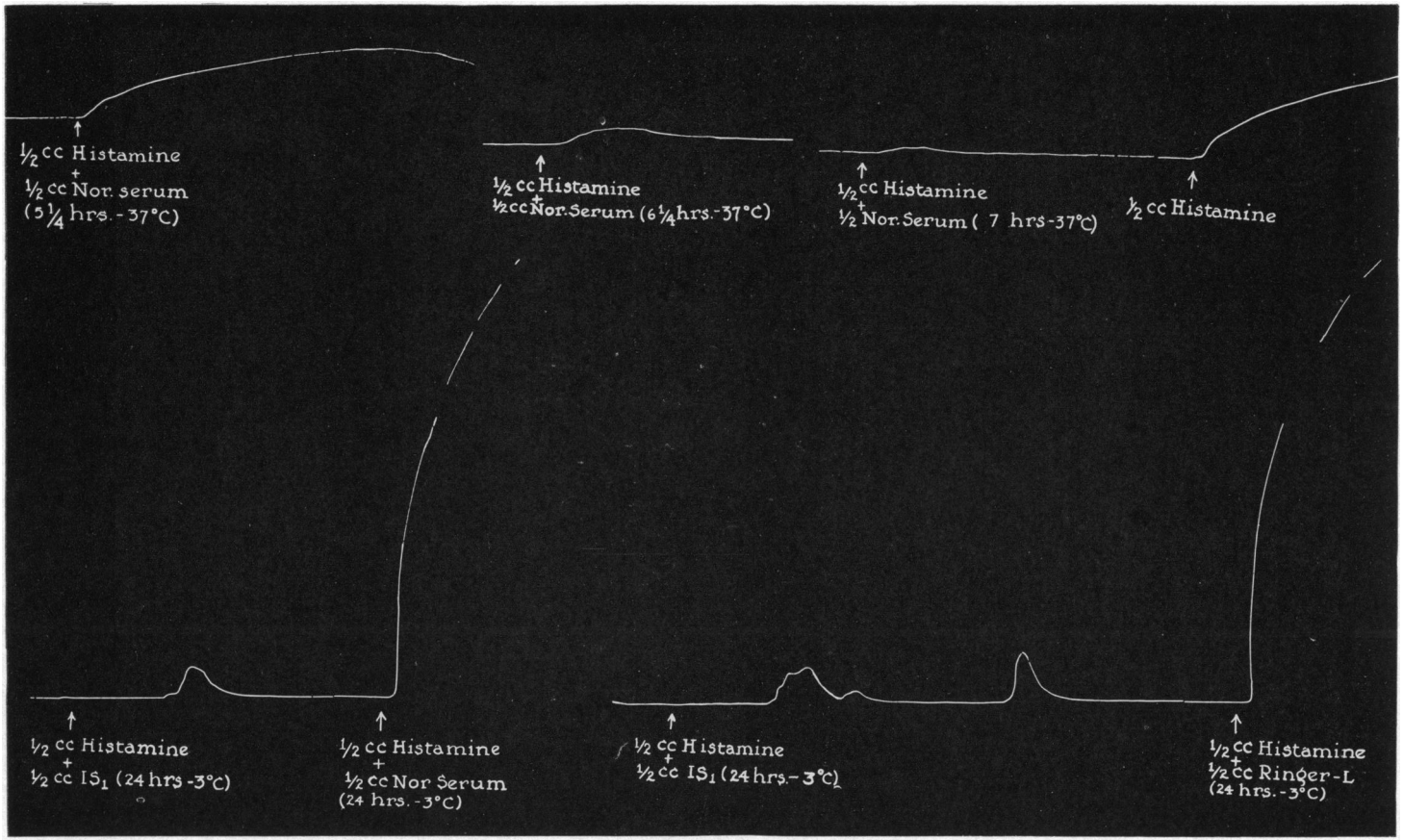
Normal Serum.—The mixing of histamine with normal pig serum did not decrease the action of the former unless kept overnight at room temperature (30° C. to 34° C.) or seven hours at 37° C. (Charts 1 and 2). At ice-box temperature (2° C. to 4° C.) contraction seemed to be enhanced (Chart 3). Heating the normal serum at 60° C. for half an hour did not destroy its neutralizing action. On the contrary, it was seemingly increased. Normal, citrated blood, heated or unheated, mixed with histamine for five to five and one-quarter hours (37° C.), or if incubated and then kept in an ice-box for 24 hours, did not neutralize the action of histamine as determined by the muscle strip. Serum under the same conditions was counteractive (Table I).

Burned Animals.—The serum of burned animals in which a histaminoid substance was still detected in moderate quantities failed to neutralize the action of histamine. Similarly, if the histaminoid action was destroyed by heating at 60° C. for half an hour, histamine neutralization was not demonstrable. Shortly after the burn, with the disappearence of the histaminoid substance, counteracting substances were still absent. Neutralizing bodies were contained in the serum of previously burned animals in whom the wounds had completely healed. If the latter serum was mixed with histamine for five and one-half hours at incubator temperature or kept in the ice-box or at room temperature overnight (Chart 3), the activity of histamine as determined by the muscle strip was diminished to absent (normal serum kept in the ice-box overnight failed to neutralize histamine (Chart 3).

Human serum thus far examined failed to neutralize histamine when applied to the smooth muscle strip.

Histamine itself in 1:100,000 dilutions, allowed to stand from one to 24 hours at room temperature, or incubated (37° C.) for from ten minutes to five and one-half hours, or at ice-box temperature (2° C. to 4° C.), or in any of the above combinations, did not show a decrease in its activity (Table I).

SUMMARY.—Normal pig serum has a limited ability to counteract or neutralize histamine in the dilutions used and under the conditions of the experiment. This action is possible only if the two substances are incubated for a long period of time. Serum of burned animals or humans in which histaminoid substances were still detectable failed to counteract histamine under the same conditions. The serum of recently burned animals



CHARTS 1, 2, 3.—The action of normal serum and the serum of burned pigs on histamine.

not containing histaminoid substances also failed to counteract the action of histamine, while in the older burned animals their serums had this neutralizing ability. This activity could be appreciated at incubator temperature (37° C.) or at ice-box temperature (2° C. to 4° C.). Heating at 60° C. for half an hour did not alter this activity.

II. Counteracting Burn Toxin.—Normal Serum.—The sources of the histaminoid substances are given in Table II. It appears that normal serum has a neutralizing action upon histaminoid substances only if the mixtures are incubated for a long period of time (Chart 4). Ice-box incubation is without effect. In one instance neutralization was not effective, but repeating it after two days gave positive results. Aging did not, as a rule, decrease the histaminoid activity, but it did enhance, in certain cases, the neutralizing ability of the serum. Contamination, as will also be noted later, impairs the neutralizing powers. The adult pig serum containing histaminoid substances was not neutralized by the normal adult pig serum. (Incubation only ten to 30 minutes.)

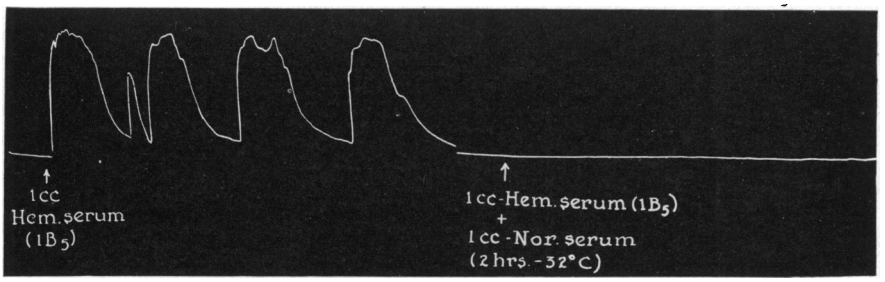


CHART 4.—The action of normal pig serum on burn toxin of shoat.

Human serum containing histaminoid substances was not counteracted by normal human serum under the conditions of the experiment (ten to 35 minutes at 37° C.).

Serum in Burned Cases.—Pigs.—The serum, red blood cells, or citrated blood of burned shoats containing histaminoid substances was not acted upon by the serum or citrated blood of adult pigs that had been burned previously but that still contained histaminoid substances. By destroying the latter activities by heating, neutralizing properties could not be demonstrated. The serum of adult burned pigs not containing histaminoid substances neutralized the action of the burn toxin of recently burned shoats and adult pigs (Chart 5). The serum of a shoat burned 20 days previously in whose serum no histaminoid substances could be detected counteracted burn toxin in adult pigs to the extent of 0.05 cc. of the former to 1 cc. of the latter (Charts 6 and 7). Normal adult pig serum under the same conditions (using the same uterus preparation) did not counteract the above in 1 cc. amounts. The above neutralizing serum when mixed with the serum of a recently burned shoat containing large quantities of histaminoid substances reduced the latter's action to one-half (Table III).

TABLE II

THE ACTION OF NORMAL PIG AND HUMAN SERUM ON HISTAMINOID SUBSTANCES

Source	Type of Material	Time after Burn	Presence of Histaminoid Substances	Source of Normal Serum	Presence of Histaminoid Substances	Reaction of Mixture on Virgin Guinea-Pig Uterus
Shoat	S.	19 hrs.	+ + +	Pig	o	+ + + (48 hrs. 3° C.)
Shoat	Cit. b.	19 hrs.	tr.	Pig	o	o (5 hrs. 37° C.)
Shoat	S.	4 da.	+ + + +	Pig	o	+ + (1¾ hrs. 37° C.)
Shoat	S.	5 da.	+ + +	Pig	o	o (2 hrs. 37° C.)
Shoat	R.B.C.	5 da.	+ +	Pig	o	o (3 hrs. 37° C.)
Shoat	Cit. b.	5 da.	tr.	Pig	o	o (3 hrs. 37° C.)
Shoat	S.	9 da.	+ +	Pig	o	+ + (2 hrs. 37° C.)
Shoat	S.	9 da.	+ +	Pig	o	tr.* (1 hr. 37° C.+ 1 hr. 32° C.)
Shoat	S.	14 da.	+ +	Pig	o	tr.
Pig	S.	2 mos.	+	Pig	o	+ (30 min. 37° C.)
Pig	S.	2 mos.	+	Pig	o	+ +
Human	S.	37 da.	+	Human	o	tr.
Human	S.		+	Human	o	+ (35 min. 37° C.)
Human	S.	34 da.	+ +	Human	o	+ +
Human	S.	27 da.	+	Human	o	+

* Repeated two days later.

+ + = action of 1 cc. of a 1 :100,000 histamine solution; S. = serum; Cit. b. = citrated blood; R.B.C. = red blood corpuscles; tr. = trace; where not indicated, incubation (37° C.) five to ten minutes.

TABLE III

THE ACTION OF VARIOUS SERUMS ON THE BURN TOXIC

Source	Type of Material	Time after Burn	Presence of Histaminoid Substances	Source of Counteractant	Type of Material	Time after Burn	Presence of Histaminoid Substances	Reaction of Mixture on Virgin Guinea-Pig Uterus
								+ + + (24–48 hrs. 3° C.)
Shoat	S.	19 hrs.	+ + +	Pig	S.	2 mos.*	+ + +	+ + + (5 hrs. 37° C.)
Shoat	S. Cit. b. R.B.C.	5 da.	+ + + tr. + +	Pig	S. Cit. b.	2 mos.*	+ + +	+ + + (24–48 hrs. 3° C.) + + + (5 hrs. 37° C.)
Shoat	R.B.C.	2 hrs.	+	Pig	S.	2 mos.	o	+
Shoat	S.	4 da.	+ + + +	Pig	S.	2 mos.	o	+
Shoat	S.	9 da.	+ +	Pig	S. old cont.	2 mos.	o	+ +
Shoat	S.	9 da.	+ +	Pig	S.	2 mos.	o	tr. (1 hr. 37° C.)
Shoat	S.	14 da.	+ +	Pig	S. (h)	2 mos.	+ + o (h)	+ + +
Pig	S.	2 mos.	+	Pig	S.	2 mos.	o	o (30 min. 37° C.)
Pig	S.	2 mos.	+	Pig	S.	2 mos.	o	+
Pig	S.	2 mos.	+	Shoat	S.	20 da.	o	o
Pig	S.	2 mos.	+	Shoat	S.	20 da.	o	o
Pig	S.	2 mos.	+	Shoat	S. 0.3 cc.	20 da.	o	o
Pig	S.	2 mos.	+	Shoat	S. 0.05 cc.	20 da.	o	o
Shoat	S.	4 da.	+ + + +	Shoat	S.	20 da.	o	+ + (1 hr. 37°C.)
Human	S.	27 da.	+ +	Human	S.	13 mos.	tr.	o
Human	S.	34 da.	+ + +	Human	S.	13 mos.	tr.	tr.

* Biopsy sites not healed.

+ + = action of 1 cc. of a 1 :100,000 histamine solution; S. = serum; Cit. b. = citrated blood; R.B.C. = red blood corpuscles; tr. = trace; cont. = contaminated; (h) = heated.

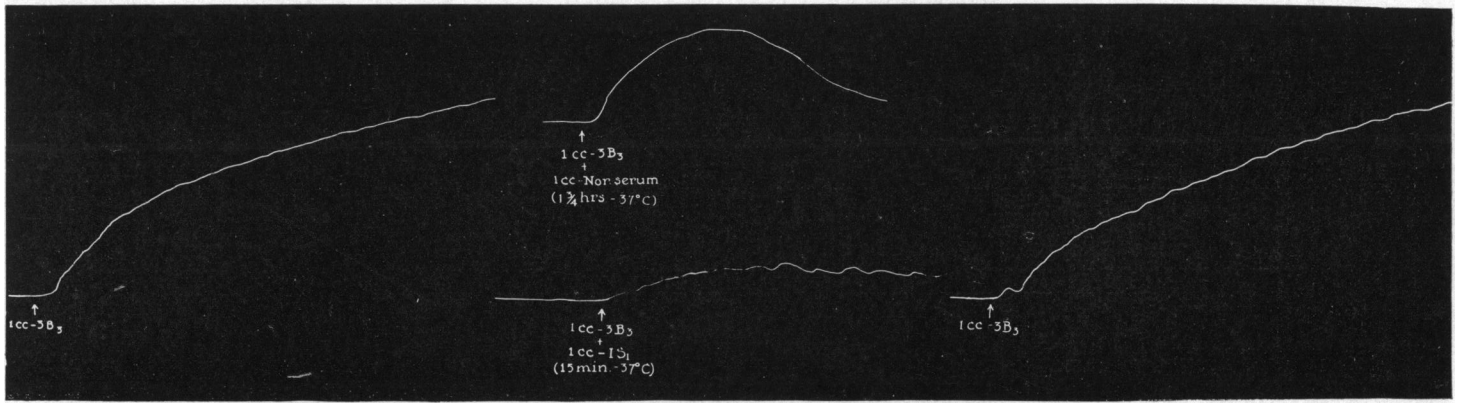


CHART 5.—The action of healed burned pig serum (IS_1) on burn toxin of shoat ($_3B_3$).

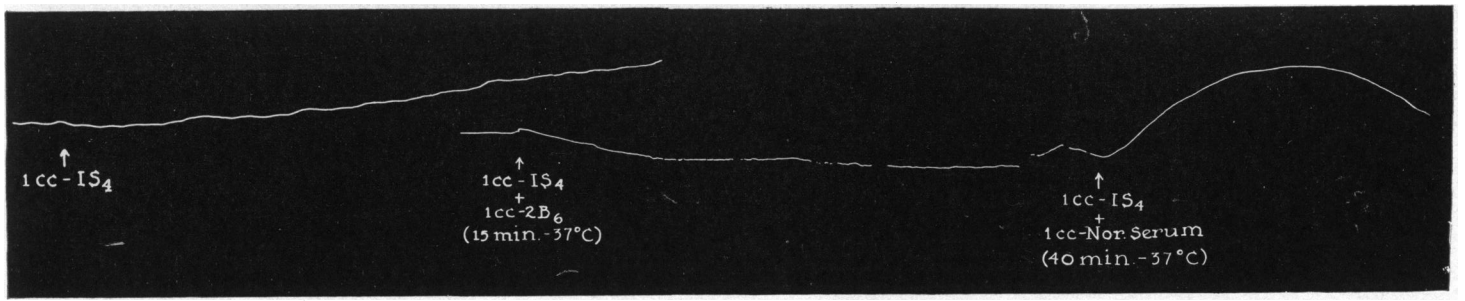


CHART 6.—The action of healed shoat serum ($_2B_6$) on burn toxin of pig (IS_4).

Human.—The serum of a burned human (13 months after extensive burning of the anterior surface of the body which had left numerous keloids and whose skin graft had become recently healed) neutralized human serum containing histaminoid substances (Chart 8). The tracings obtained with human specimens were strikingly similar to those obtained with the animal specimens (Chart 5). It is to be noted that under the same conditions, normal human serum had no effect.

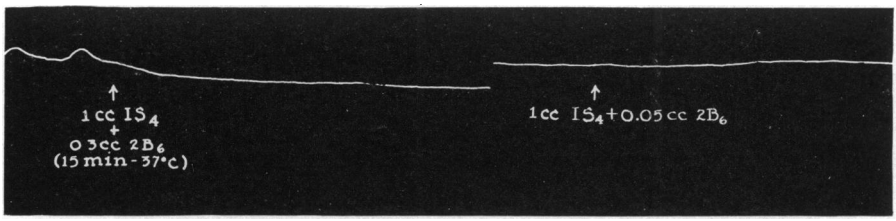


CHART 7.—The action of healed shoat serum ($2B_6$) on burn toxin of pigs (IS_4).

Contamination of the antihistaminoid containing serum nullified its neutralizing qualities. Aging, on the other hand, enhanced it. In rare instances, histaminoid substances became refractive to normal or burn toxin neutralizing serums.

SUMMARY.—Certain burned adult pig or shoat serums, in which detectable smooth muscle contracting substances have disappeared, have the ability to counteract the burn toxin of burned shoats or pigs, as determined by its activity on the virgin guinea-pig uterus. The same holds true for human serums.

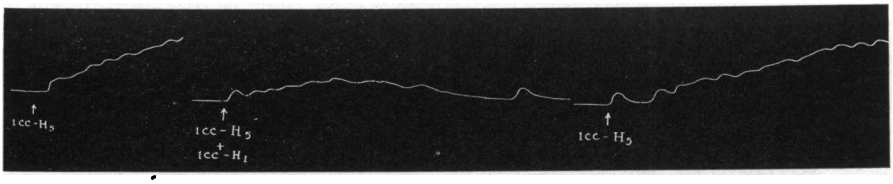


CHART 8.—The action of healed burned human (H_1) on burn toxin of human (H_5).

Normal serum of either man or animal has definite histaminoid neutralizing properties but only if incubated (37° C.) over a long period of time.

Discussion.—In a previous publication,[11] the author has demonstrated that the serum of burned animals and humans contained histaminoid substances. The present work seems to indicate that the serum in cases of healed burns (humans or animals) contains neutralizing substances for both humans and animals, respectively, as determined by the action of the mixture on the virgin guinea-pig uterus. Normal serum, too, possesses this property to a limited extent. (The failure of Busson and Kirschbaum[5] to neutralize histaminoid substances by shaking with normal serum may be accounted for by the fact that they did not incubate the mixtures at 37° C.)

In anaphylaxis, as in allergic states (bacterial, food, pollens, *etc.*), where antigen and antibodies are supposedly involved, histaminoid substances, according to the latest theories, play an active rôle (Aronson,[1] Lewis and Grant,[8] McConnell[9]). Thus, the histamine wheal in allergic states simulates the allergin wheals (Lewis and Grant[8] and McConnell[9]), and in acute anaphylaxis histaminoid substances are markedly increased in the lung (Feldberg and Schilf).

The existence of neutralizing properties against histamine in the body has been denied by Feldberg and Schilf, who believe, rather, that the liver and the lung remove the histamine from circulation. Adrenalin will reduce the histamine skin wheal, and if injected at the same time no wheal will develop (Mogena and Fernandez[10]). The blood pressure fall in histamine shock can be elevated to normal by adrenalin (Chen[6]). Barium chloride will also do the same, but the plasma exudation and the increase of hemoglobin concentration is not affected (Underhill and Ringer[13]). Best and McHenry[3] have suggested the presence of an histaminase, for in the autolysis of tissues, histamine disappears, but if the autolysate is heated at 95° C. for four minutes, the histamine persists.

Histamine-like substances found in burns are not influenced by adrenalin, hypophyseal extract or grapesugar solution, according to Feldberg and Schilf.

SUMMARY.—Under the conditions of the experiment: (1) There are indications that the serum of healed burned shoats, pigs and humans contained substances which will neutralize histamine and burn toxin, as determined by the action of the mixture on the virgin guinea-pig uterus. This reaction takes place at room (30° C. to 34° C.), ice-box (2° C. to 4° C.), and incubator temperatures (37° C.). Heating at 60° C. for half an hour does not destroy this action. (2) Normal serum also neutralizes histamine and burn toxin to a limited extent, and then only at incubator temperature (37° C.) for a period of time.

I wish to thank Drs. Bernard Fantus and Lloyd Arnold for their interest in this work.

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