from animal to animal is the same for tissue slices in an atmosphere of oxygen as for the total metabolism of the intact organism.

c. The control is intrinsic in the sense that the relation to body weight of the respiration of the excised tissue slices parallels the metabolism of the intact animal, at least during the one or 2 hours required by the Warburg technic. If the control is ultimately central, as by some hormonal regulation of the concentration of respiratory enzymes, the peripheral "set" involved must have a large measure of inertia, as it is not readily altered by excision and the disruption of all systemic channels of control.

d. Through a striking range the intensity of respiration is regulated between tissue and tissue and from animal to animal so as to present the following picture: The relative intensity of respiration is proportional to the relative body weight of the animal, while the tissues preserve their relative relations. As a result the plot of the log oxygen consumption on the log body weight (thus placing both on a relative basis) yields a family of parallel straight lines, one for each tissue. This conclusion is necessitated not only by the relation shown above for the Q_{O_2} of kidney, liver and brain but also by the fact that the total metabolism of a series of animals parallels the Q_{O_2} of any one of the tissues for these same animals.

13568

Agglutination of Rabbit Leucocytes by *Staphylococcus aureus* Toxin.

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Several years ago, while testing streptococcal-extract toxin[1] for leucocidin by the Neisser-Wechsberg technic, one of us noted agglutination of the leucocytes in some of the tubes. Such agglutination appeared only on dilution of the toxins.

Recently a similar phenomenon was observed with staphylococcal toxin. This paper deals with a method developed for testing of staphylococcal toxin for these leuco-agglutinins.

Methods. Most of this work was carried out with the Wood strain

[1] Weld, Julia T., *J. Exp. Med.*, 1934, **59**, 83.

of staphylococcus. However, similar results have been obtained with several other strains.

The medium used was a phosphate-buffered veal-infusion broth,[1] with proteose-peptone, containing 1-20,000 phenol-red indicator (Merck). This medium was used for the growth of the organisms in the preparation of the toxins and, without peptone, for the suspension of the leucocytes and the dilution of the toxins used in the tests.

The organisms were grown for 4 or 5 days in 50 cc Erlenmeyer flasks containing 25 cc of medium and 0.5 g of cellophane.[2] The cultures were then centrifuged, filtered at pH 8 through Berkefeld V filters and stored at pH 7.2 in the ice box, usually under vaseline seals.

The leucocytes were obtained from young rabbits weighing 1500 to 2000 g following the intrapleural injection of 20 cc of saline containing 5% aleuronat and 3% starch. Eighteen hours after injection the animals were bled to death from the carotid and the pleural exudates taken up in 1/10 their volume in freshly prepared 1-100 heparin in distilled water. Exudates containing a small number of red cells may be used in these tests. The exudates from both pleural cavities were combined, mixed, and distributed in 5 cc quantities in test tubes in which they were centrifuged slowly (about 10 minutes) until the supernatant suspensions were almost clear. The leucocytic sediments were combined and suspended in broth at pH 7.2 so that each cu mm contained 20,000 leucocytes. The leucocytes were kept warm during the preparation of the suspensions and subsequent tests.

The agglutinative test as finally developed was carried out as follows: 0.2 cc of toxin in various dilutions and 0.2 cc of control broth were pipetted separately into a series of tubes 10 x 75 mm. The freshly prepared leucocytic suspension was then pipetted into each tube in 0.2 cc quantities. The tubes were shaken immediately and placed in a water-bath at 37°C for 1½ hours, during which time each rack was taken out and shaken at intervals of 15 minutes. Following this the tubes were placed in the icebox at 4°C for 30 minutes and then read for leuco-agglutinin.

Results. Our results were always consistent when care was taken to prepare the leucocytic suspension exactly as described above.

Agglutination first appears in 1 or 2 of the tubes after approximately 50 minutes of incubation. The time of first appearance of agglutination is constant for any one filtrate. After further incubation the zone of agglutination spreads gradually into the tubes con-

[2] McClean, Douglas, *J. Path. and Bact.*, 1937, **44**, 47.

TABLE I.
Agglutination of Leucocytes by Staphylococcal Toxin.

	Strain of organism	Dilution of toxins undil.	1-2	1-5	1-10	1-20	1-50	1-100	1-200	1-400	Control broth
A	Wood	0	0	0	+	+++	++++	++++	++	+	0
B	103	++++	++++	++	+	0	0	0	—	—	0

+ to ++++ indicates varying degrees of agglutination.
++++ indicates complete agglutination.
0 indicates no agglutination.

taining diminishing amounts of toxin and, in the case of strong toxins, into the tubes containing increasing amounts of toxin. After 1½ hours' incubation, the leucocytes are clumped usually in one large gelatinous clot in the one or two tubes in which agglutination first appeared, leaving the surrounding fluid absolutely clear. The agglutinated clots are not broken up by shaking. A prozone always occurs unless the toxin is weak in agglutinative action.

In Table I are given the titrations of 2 toxins for leuco-agglutinins, A, a strong toxin showing the prozone effect and B, a weak toxin showing no prozone.

For purposes of calculation 1 unit of leuco-agglutinin represents the minimal amount of toxin that causes complete agglutination of leucocytes after 1½ hours at 37°C and 30 minutes in the icebox.

If films of the suspensions after incubation are prepared with Wright's stain, it is seen that the leucocytes in the prozonal tubes are completely disintegrated. In fact, when examined macroscopically, these tubes appear less opaque than the control tube of leucocytes and broth. This suggests that living intact leucocytes are necessary for agglutination to occur, which is borne out by the fact that for clear-cut results in these tests, it is important to use fresh leucocytic suspensions. Stains of smears made from the gelatinous clots recovered from the tubes with maximal agglutination show an amorphous blue-staining substance in which leucocytes can be made out only as faintly stained forms without demonstrable nuclei.

Correlation of leuco-agglutinins and leucocidins. Tests for leucocidins were carried out according to the "bioscopic" method of Neisser and Wechsberg with certain modifications. In order to compare exactly the leuco-agglutinin- and leucocidin-content of staphylococcal toxins, dilutions of toxins and leucocytic suspensions as described before were set up in duplicate. After 1 hour at 37°C both series of tubes were read for agglutination of leucocytes. Then one series of tubes was placed in the icebox and 0.05 cc of 1-10,000 methylene blue (Special Methylene Blue—LaMotte Chemical Co.)

and vaseline seals were added to each tube in the second series. The methylene blue tubes were then incubated overnight, this long incubation at 37°C being necessary in order to obtain complete reduction in the control tubes of leucocytes and broth. The medium used was always controlled for possible reduction by itself by incubating a tube of broth with methylene blue along with the other tests.

These tests showed a definite correlation between the amount of leucocidin and leuco-agglutinin in the toxins. There was regularly no reduction of methylene blue in the prozonal tubes nor in those showing complete agglutination, indicating that the leucocytes in these tubes had been killed. There was partial reduction usually in the tubes showing a ++ or +++ agglutination and complete reduction in the other tubes including the leucocyte and broth control. The control broth alone has never shown any reduction. Leuco-agglutinin and leucocidin also show a similar susceptibility to heat, both activities being destroyed when staphylococcal toxin is heated to 56°C for 1 hour. These results appear to indicate that staphylococcal leuco-agglutinin and leucocidin are closely related if not identical.

Agglutination of Rabbit Bone-marrow by Staphylococcal Toxin. Since, as has been demonstrated, staphylococcal toxin has an agglutinative effect on rabbit leucocytes, it seemed possible that it might also have a similar effect on rabbit bone-marrow. We found that such was the case. Exactly similar results in all respects were obtained when tests were run using bone-marrow suspension instead of leucocytic suspension and staphylococcal toxin.

In brief, the method of preparing bone-marrow suspensions is as follows: marrow obtained from the femur of a normal rabbit is quickly ground without sand in a warm mortar, and is taken up and emulsified in warm broth. Special care must be taken to keep the marrow warm throughout the entire process of preparing the suspension, otherwise a homogeneous suspension is not obtained and must be discarded. The suspension is pipetted with a warmed pipet into the tubes containing the toxin-dilutions which are standing in the water bath at 37°C.

Discussion and Summary. This report deals with an agglutinating reaction of staphylococcal toxin for rabbit leucocytes. When this test is carried out carefully, the results are clear-cut and remarkably consistent. Except for the fact that the leucocytic suspension is more exacting than a red cell suspension to prepare, the test is as easily and quickly set up and read as a test for bacterial hemolysin.

Our experiments demonstrate that there is a definite correlation between the content of leuco-agglutinin and the content of leucocidin

in any toxin, also that both activities are destroyed at the same temperature.

If we are correct in the interpretation of our results, leuco-agglutinin and leucocidin actually represent two stages of the action of the same toxic substance, and the leuco-agglutinin test, because of its simplicity and its clear-cut quantitative results, may be used to advantage over previous methods for the quantitative determination of leucocidin in staphylococcal toxins.

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Effect of Propazone on Respiration of Rat Tissue *in vitro*.*

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Propazone (5, 5-di-n-propyl-2, 4-oxazolidinedione) is one of a new series of compounds recently prepared and studied by Stoughton,[1] which has been shown to have hypnotic and anesthetic properties.[2] The structural relationship to the barbiturates and hydantoinates indicates that this agent might inhibit tissue respiration as do these other fixed hypnotics.[3] Propazone sodium† offers certain advantages in this type of study in that it is more soluble in water than the barbiturates and is nearly neutral in reaction. The effect of propazone on the respiration of rat liver, kidney cortex and cerebral cortex slices is described in the present paper. Twenty-four adult male rats were used.

The rate of oxygen consumption was measured by the Warburg manometric method, and is expressed in μl, N.P.T., per mg wet weight per hour (Q_{O_2}). The methods of preparation of tissue slices have been described previously.[4] The suspension medium was Ringer's phosphate, pH 7.35, containing 0.2% glucose. Propazone sodium was dissolved in glucose-free Ringer's phosphate and added

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1 Stoughton, R. W., *J. Am. Chem. Soc.*, 1941, **63**, 2376.

2 Stoughton, R. W., and Baxter, J. H., *J. Pharm. and Exp. Ther.*, 1941, **73**, 45.

3 Quastel, J. H., *Physiol. Revs.*, 1939, **19**, 135.

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4 Fuhrman, F. A., and Field, J., 2d, *J. Pharm. and Exp. Ther.*, in press.