for acetone dried *S. typhosa* Ty2 are as follows: *E. coli,* 6; *P. ballerup,* 3; *S. paratyphi* C, 0.02. It thus appears that *E. coli* 5396/38 is the richest source of Vi antigen among the known V form *enterobacteriaceae.*

1. Felix, A., and Pitt, R. M., *J. Hyg.,* 1935, v35, 428.

2. Henderson, D. W., *Brit. J. Exp. Path.,* 1939, v20, 1.

3. Kauffmann, F., and Moller, E., *J. Hyg.,* 1940, v40, 246.

4. Longfellow, D., and Luippold, G. F., *Am. J. Hyg.,* 1943, v37, 206.

5. Kauffmann, F., *Die Bakteriologie der Salmonella-Gruppe* (Typhus-Paratyphus-Enteritis-Gruppe). Ejnar Munksgaard. Copenhagen, 1941.

6. Landy, M., *Pub. Health Rep.,* 1950, v65, 950.

7. Henderson, D. W., Amies, C. R., and Steabben, D. B., *Rep. Lister Inst. Prev. Med.,* 1940, 7.

8. Luippold, G. F., *Am. J. Pub. Health,* 1946, v36, 15.

9. Batson, H. C., *J. Exp. Med.,* 1949, v90, 233.

10. Knudsen, L. F., and Curtis, J. M., *J. Am. Stat. Assn.,* 1947, v42, 282.

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Failure to Demonstrate *in Vitro* Lysis of Sensitized Guinea Pig Leucocytes by Dog Hemoglobin Antigen.* (19521)

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Squier and Lee(1) have reported demonstration of a mean average reduction of 43% in the total number of leucocytes resulting from the addition of short ragweed antigen to heparinized whole blood from patients sensitive clinically and by skin test to the ragweed pollen. This reduction affected chiefly the polymorphonuclear leucocytes. It was postulated that the leucocyte reduction was due to an antigen-antibody reaction. It was further postulated that the antibody in this instance was a thermolabile reagin inactivated by heating to 56°C. Franklin and Lowell have failed to duplicate these results(2). This report describes an attempt to apply Squier and Lee's technic to a study of some phases of hypersensitivity and anaphylactic shock, using mature guinea pigs.†

Method. The hemoglobin suspension utilized as antigen in this experiment was prepared from citrated whole blood of dogs. The erythrocytes were separated by centrifugation and washed 3 times with normal saline, following which they were lysed with distilled water. Sufficient 18% NaCl was added to make a slightly hypertonic solution and again centrifuged to precipitate the stroma of the erythrocytes. The supernatant was drawn off and to this added about ⅓ its volume of "Amphogel". After standing for about 2 hours this latter mixture was filtered through coarse filter paper. The filtrate contained nearly pure hemoglobin and was utilized as antigen in the experiment. Twelve guinea pigs ranging in weight from 300 to 400 g were sensitized by 2 subcutaneous injections of 0.2 ml portions of the hemoglobin antigen at 48-hour intervals. Ten control guinea pigs ranging in weight from 300 to 350 g were similarly injected with 0.2 ml portions of normal saline. In the experimental group 5 females and 7 males were used. In the control group 5 females and 5 males were used. These animals were caged in groups of 5 and the sexes kept separated. None of the females were known to be pregnant. The diet was considered adequate and all of the animals were apparently healthy. In the control group blood was drawn by intracardiac puncture on the 13th or the 15th day after the second subcutaneous dose of saline. In the experimental group blood was similarly drawn on the 20th, 21st, or 32nd day after the second sensitizing dose of hemoglobin. The experi-

* This study was carried out under contract with the Division of Biology and Medicine, U.S.A.E.C.

† Animals obtained from Carworth Farms.

mental animals were subsequently subjected to 1.0 ml intracardiac doses of the antigen to prove sensitivity. The blood removed from each guinea pig was heparinized and divided into equal portions in 2 Wassermann bottles. To the first portion was added normal saline and to the second hemoglobin antigen. The concentration of blood to antigen varied from 2:0.1 to 3:0.1 ml. In order to rule out the possibility of insufficient concentration of antigen, an additional 0.4 ml of antigen was added to the blood specimens from the last 2 animals after 120 minutes, increasing the concentration of blood:antigen in these instances to 2.6:0.5 ml. In each instance the ratio of blood:saline was the same as that of blood: antigen. The tubes were rotated vigorously to mix and white cell counts made immediately after mixing blood and saline or blood and antigen, and repeated at 30, 60, and 120 minutes from each tube. With the last 2 experimental animals counts were also made at 180 and 240 minutes during which time a significant change did not occur. In each instance 2 counts were made at each interval and the results recorded represent averages of these counts. Diluted blood specimens were counted within a few minutes of being drawn into the pipettes. The time interval between cardiac puncture and the addition of the saline or antigen to a given specimen was usually 1 to 2 hours. In the case of the last 2 experiments, however, the delay was 15 and 20 minutes respectively.

Results. Blood from control and sensitized animals showed no leucocyte lysis when tested *in vitro* with the hemoglobin antigen. The interval of time elapsing between sensitization and attempted *in vitro* shock had no detectable effect on results. Concentration of antigen in the range studied likewise did not significantly alter results. No sex differences were noted.

It was found that the mean white cell count of the sensitized guinea pigs made immediately after mixing in the tubes with blood and saline is 32.7% above that of the non-sensitized pigs. In the tubes with blood and hemoglobin antigen the mean white cell count of the sensitized guinea pigs is 34.0% above that of the non-sensitized pigs. It was not determined whether the higher white cell counts of the sensitized animals was due to the presence of eosinophils.

TABLE I. Total Leucocyte Counts on Heparinized Blood from 10 Control Guinea Pigs.

Sex	Blood + normal saline			
	Immed.	30″	60″	120″
♀	6500	6225	5950	5525
♀	5225	5650	5475	5425
♀	3450	3525	3150	2950
♀	7625	7525	6650	5975
♀	3350	3450	3825	3175
♂	3600	4200	3625	3825
♂	5600	5500	6050	5325
♂	4350	3950	3425	3950
♂	5000	5400	6150	5600
♂	5600	5975	5450	4550
Mean	5030	5140	4975	4630
	Blood + hemoglobin antigen			
♀	6675	5875	6925	5975
♀	4725	5325	5475	6075
♀	3725	3025	3200	3150
♀	6675	7050	6475	6175
♀	2725	3450	3550	3850
♂	2925	2500	3000	2975
♂	4925	4950	5225	5225
♂	4750	4600	3850	4250
♂	4475	6675	5600	4875
♂	5450	4375	5025	3225
Mean	4705	4783	4833	4578

Of the experimental animals in this series 2 died of hemorrhage incident to intracardiac puncture. Of the remaining 10 animals, fatal anaphylactic shock was induced in 7 on injection of 1.0 ml of hemoglobin antigen. There was mild shock in one animal and no shock was apparent in two. These results are tabulated in Tables I and II.

Discussion. The coefficient of variation for white cell counts made from a single blood specimen as reported by Berkson is 10.7% (3). A criticism of inadequate sampling with routine technic would therefore be valid. It is doubtful, however, that errors inherent in the counting technic could obscure a mean reduction of 43% in the total white cell counts were it present, or show one that is not present.

Seven of the 10 animals subjected to the shock dose of hemoglobin antigen died immediately of typical anaphylactic shock. This demonstrates that the majority of the experimental animals in this series were highly sensitive to the antigen employed.

The significance of the increased mean white cell count of the sensitized guinea pigs over

TABLE II. Total Leucocyte Counts on Heparinized Blood from 12 Guinea Pigs Sensitized with Hemoglobin Suspension.

Sex	Days inc. (*In vitro* shock)	Blood + normal saline				Blood + hemoglobin antigen				Days inc. (*In vivo* shock)	Result shock dose
		Immed.	30″	60″	120″	Immed.	30″	60″	120″		
		3650	3100	2800	2975	2525	3275	3250	2575	Acc. hemorrhage	
		6325	5725	7200	6275	5450	5350	5250	5300		
♀	20	3225	3525	4375	3700	3125	2925	3425	3300		
		6325	6075	6625	6475	6075	5025	5600	5575	25	Fatal
		10550	8400	9550	9375	10200	9425	8475	8625		
		7675	8000	7650	8325	8350	7675	7350	8225		
		8425	7125	7600	7400	8350	7975	7425	8000		
♂	21	8500	7325	6250	6475	7375	7100	7225	6700	25	No shock
		5550	5375	6200	6525	5175	5725	6250	5950		
		8450	9700	9125	10325	8225	9150	9150	9275	Acc. hemorrhage	
♂*(1)	32	5825	6275	5525	6500	5550	4950	5950	5550	32	Fatal
♂*(2)	32	5575	4825	5500	5025	5275	5150	4825	4525	32	Mild shock
	Mean	6673	6288	6536	6615	6306	6144	6181	6133		

* Last 2 guinea pigs counted 2 additional hr. (Additional .4 ml saline and antigen added to respective tubes.) Values follow:

	Blood + normal saline		Blood + hemoglobin antigen	
	180″	240″	180″	240″
(1)	5025	4975	4325	4700
(2)	4625	4525	4150	4150

the non-sensitized pigs as related to leucocyte lysis in shock is not clear.

Summary and conclusions. Twelve guinea pigs were sensitized with dog hemoglobin. Subsequently blood specimens were taken and heparinized and a portion of these specimens mixed with the antigen. White cell counts were done immediately after mixing, and repeated at 30, 60, and 120 minutes. In 2 cases counts were also made at 180 and 240 minutes. Controls of blood and saline in the same ratio as blood and antigen were counted concomitantly on each guinea pig. Blood specimens from 10 non-sensitized guinea pigs were similarly collected and counted. No significant difference in the total white cell counts from sensitized or non-sensitized animals either when mixed with saline or with the hemoglobin antigen was detected. The total mean white cell count of the sensitized animals in this series was 32.7% and 34.0% above that of the non-sensitized animals in the control and experimental tubes respectively. The above findings indicate that *in vitro* lysis of sensitized guinea pig leucocytes by dog hemoglobin antigen does not occur.

1. Squier, T. L., and Lee, H. J., *J. Allergy,* 1947, v18, 156.

2. Franklin, William, and Lowell, Francis C., *J. Allergy,* 1949, v20, 375.

3. Berkson, J., Magath, T. B., and Burn, M., *Am. J. Phys.,* 1940, v128, 309.

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Effect of Electroconvulsive Shock on Adrenal Cortex of the Rat.* (19522)

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