

periments will have to show whether the test is specific for the poison or due to an accompanying impurity.

Summary. A simple and efficient method for the purification of shellfish poison consists in differential retardation when the poison is filtered through a charcoal column. The resulting products are low in ash, have lost their fluorescence, have an equal number of Cl and N atoms and give a characteristic Nessler reaction.

After completion of this work the article by Tiselius (*Science*, 1941, **94**, 145) came to our attention in which the author demonstrated the differential retardation of various salts, sugars, amino acids and peptides through active carbon and other adsorbents and proposes the procedure as a method of analysis of wide application. The separation of the colorless substances is, in this case, made visible by the Schlieren method.

13437 P

Production of Anemia by Tetanus Toxin.

HANNAH FARKAS AND I. J. KLIGLER.

From the Department of Hygiene and Bacteriology, Hebrew University, Jerusalem.

Until the recent extensive studies by Abel¹ it was generally accepted that tetanus toxin reaches the central nervous system by way of the nerve tracts. This view was based largely on the experimental work of Marie, Marie and Morax and others.² However, the experiments of Abel and his associates¹ showed that the toxin was distributed through the body by way of the lymphatic and blood systems, the specific effect of the toxin being produced on the nerve tissue with which it came in contact. Even prior to Abel's work, Van den Reis³ according to Weinberg suggested that tetanus toxin caused a secondary anemia.

The object of the experiments reported below was to study the effect of tetanus toxin on the blood. The experiments were made on rabbits which in contrast with guinea pigs, rats and mice gave a constant red cell count. Individual normal rabbits examined regularly during a period of one month showed only slight variations in total

¹ Abel, J. J., *Science*, 1934, **79**, 63, 121; *Bull. Johns Hopk. Hosp.*, 1935a, **56**, 84, 317; 1935, **57**, 343.

² Marie, A., *Ann. Inst. Pasteur*, 1897, **11**, 591; 1898, **12**, 91; Marie, A., and Morax, V., *Ann. Inst. Pasteur*, 1902, **16**, 818; Meyer, H., and Ransom, F., *Arch. Path. Pharm.*, 1903, **49**, 369.

³ Van den Reis, K. W., 1922, **1**, 950.

red cell count, hemoglobin index and sensitivity to hypotonic salt solutions.

The effect of the toxin was determined by the changes resulting in the red cell count, the hemoglobin index (Talquist), sedimentation rate and lysis, subsequent to the injection of toxin. The white cell counts showed no constant changes and were, therefore, not taken into account. Graded sublethal doses were injected intravenously or intramuscularly and the blood changes noted beginning with 1 hour after the injection and for varying periods thereafter. The potency of the toxin was titrated on mice according to Behring's method and the M.L.D. for rabbits based on VonLingesheim and Sherrington's⁴ calculations that the specific resistance of rabbits was twice that of mice.

The results of a comparable series of experiments with varying doses of toxin given i.v. and i.m. respectively, are summarized in Table I. It will be seen from the data in the table that doses as low as 0.3 M.L.D. produced marked anemia; that the degree of change was approximately the same with the doses used, but that the rate of damage was dependent on the dosage; that the same dose given i.m. acted more slowly than when given i.v. In all instances the direction of change was the same—a marked decrease in the number of red cells, 50-60%; a corresponding decrease in hemoglobin; a significant increase in the sedimentation rate.

In order to ascertain whether the anemia was due to a specific effect of the toxin, rabbits showing a marked decrease in red cells following the injection of toxin, were given an injection of antitoxin. The effect was prompt and striking; after 2 hours the number of red cells rose by 50% and after 24 hours the count was nearly normal; the hemoglobin and sedimentation results followed a parallel course.

Reticulocyte counts at different phases verified the assumption that the toxin produced a type of secondary anemia which was resolved by the injection of antitoxin. The reticulocyte counts were made by staining with brilliant cresyl blue. In one series of counts the following results were obtained:

Before injection of toxin	4.5	* reticulocytes
After injection of toxin	6.9	''
2 hours after injection of antitoxin	15.34	''
16 hours after injection of antitoxin	51	''

*Per million.

It seems that either the toxin destroys the reticulocytes as they

⁴ Lingelsheim, von. See Kolle and Wasserman's *Hdb. Path. Mikroorg.*, 1912, 2te Abt., 1912-13, 4, 737; Sherrington, C. S., *Lancet*, 1917, 2, 964.

TABLE I.

No. of exp.	Mode of injection	Dose	No. of R.B.C.		Time,* h	Hemoglobin			Sedimentation		
			At start	Minimum		At start	Minimum	Time,* h	At start, mm	Minimum, mm	Time,* h
1	i.v.	0.58	5,340,000	2,180,000	21	80	40	4.5	2	4	21
1'	i.m.	0.58	5,780,000	3,420,000	144	61	45	144	1.5	4	144
2	i.v.	0.49	4,250,000	2,000,000	46	65	45	46			
3	i.v.	0.48	3,540,000	1,100,000	2	60	35	72	2	3	48
3'	i.m.	0.47	3,750,000	2,100,000	48	65	45	48	2	3	48
4	i.v.	0.46	3,750,000	1,850,000	25	65	45	5			
4'	i.m.	0.41	5,300,000	2,940,000	144	75	50	192	2		
5	i.v.	0.3	4,600,000	1,900,000	46	75	45	25			
6	i.v.	0.27	4,200,000	2,190,000	22	65	35	22	1.5	6	22
7	i.v.	0.1	4,520,000	3,780,000	51	75	70	51	3	3.5	27
7'	i.m.	0.1	4,250,000	3,780,000	48	80	75	6	3	3	

*Time elapsed after injection when minimum was reached.

enter the blood stream or else blocks the hematopoietic system. As soon as the antitoxin is administered the reticulocytes pour into the circulation and take the place of the destroyed red cells. This accounts for the dramatic restoration of the count within two hours after the injection of the antitoxin.

While this investigation was in progress Schwarzmann⁵ showed that the toxin combines with the hemoglobin of the red corpuscles. Preliminary experiments were made to ascertain what portion of the toxin injected was present in the blood. Two rabbits were injected 13 M.L.D. of toxin, one i.v., the other i.m. Before death the rabbits were bled and the toxin content of the blood titrated by injecting varying dilutions into mice. From the results it was computed that the blood of the rabbit which received the toxin i.v. contained 40% and that of the rabbit injected i.m. 13% of the amount of toxin given.

Summary. Small sublethal doses of tetanus toxin produce a typical secondary anemia—low red cell count, reduced hemoglobin index, increased sedimentation rate. Injection of antitoxin produced a prompt return to normal, the red cell count rose rapidly as a result of large numbers of reticulocytes pouring into the system.

The severe anemia following the administration of toxin and the prompt recovery following that of antitoxin may serve as a simple early diagnostic test of possible tetanus intoxication in wounded.

⁵ Schwarzmann, G., PROC. SOC. EXP. BIOL. AND MED., 1940, 44, 112.