

not affect the oxygen consumption of activity. This is in harmony with the established differences between the carbohydrate metabolism of nerve during rest and activity.

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The Standardization of the Hemolytic Index.

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During our studies of the effect of hemolytic amboceptors on different tissues the necessity arose for the creation of a standard for the comparison of the hemolytic index of different seras. Everyone who has worked on similar problems has no doubt encountered the disadvantages of the present system of plus symbols. Not only is it subject to the personal interpretation of the investigator, but the varying degree of the hemolytic index of a given serum will depend on the age of the serum and the temperature of preservation. Furthermore the following variants of the blood cells have to be taken into consideration: Condition of the animal at the time of taking the blood, methods of preventing coagulation, differential count of the different blood-elements, volume of the red blood-cells, time of centrifugation, temperature during centrifugation, and, the most important factor of all, the increased sensitivity of the red blood cells, which rapidly increases with the time of preservation.

In order to be independent of all these variants it was thought desirable to have a standard hemolytic chemical agent, the action of which might be compared with the hemolytic effect of a given amboceptor. For this purpose sapotoxin was prepared from Quillaja bark saponin (Merck, crude), following with minor variations the methods described by Kobert and Brandl.¹ Sapotoxin has the advantage of being more effective than saponin and the methods of purification guarantee a more uniform product than the different commercial saponins.

Two systems were prepared. The first one consisted, as usual, of the hemolytic serum in varying amounts plus complement (guinea pig), plus 1 cc. of a 5% emulsion of red blood cells, which had been washed 3 times with a 0.85% NaCl solution. The second system consisted of varying amounts of sapotoxin plus 1 cc. of the same

¹ Brandl, J., *Arch. f. Exp. Path. u. Pharm.*, 1904, liv, 245.

5% emulsion of blood cells in each test tube. It had been found that a 0.05% solution of sapotoxin in a 0.85% solution of NaCl was best suited for this purpose. It was prepared in each experiment fresh from a standard 1% solution, to which 0.1% of salicylic acid had been added.

After both systems had been kept together for 2 hours in the incubator at 37°C., in both of them the test tube was determined in which complete hemolysis had occurred. It was assumed that the amount of serum which could produce complete hemolysis was equivalent to the amount of sapotoxin which had produced the same effect. For practical purposes that amount of sapotoxin was calculated in milligrams which was equivalent to the hemolytic action of 100 cc. of serum. This figure was called "Sapotoxin Units". Example: In the first system 0.025 cc. of serum produced complete hemolysis of 1 cc. of 5% blood. In the second system 0.290 cc. of the sapotoxin solution had the same effect. 0.290 cc. of a 0.05% solution of sapotoxin are equal to 0.145 mg. sapotoxin. The sapotoxin units are $0.145 \times 100/0.025 = 580$ sapotoxin units.

TABLE I.*

Sapotoxin units of different hemolytic amboceptors. Blood was injected intra-abdominally in intervals of three days. The serum was taken nine days after the last injection.

Blood from	Injected into	Amount of blood injected		Sapotoxin Units
		%	cc.	
Rat	Dog	50	3-3-3-3	95
Rat	Dog	50	4-8-12	315
Cow	Dog	50	10-10-10	800
Cow	Dog	50	10-10-10-10	1070

* E. Sieburg. In *Handbuch der biologischen Arbeitsmethoden*, E. Abderhalden Abt. I, Teil 10, pp. 545-584. 1923.

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Denaturation of Hemoglobin.

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In a recent series of papers by Anson and Mirsky,¹ the claim is made that hemoglobin, in N/20 HCl, is completely denatured in

¹ Anson, M. L., and Mirsky, A. E., *J. Gen. Physiol.*, 1930, xiii, 121, 133, 469, 477.