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A Liver Function Test for Small Laboratory Animals.

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Following studies[1] on the pharmacological behavior of certain phthalein dyes, Rosenthal and White[2] introduced bromsulphalein as an agent for the study of hepatic function. This test has been and is widely used in both clinical and experimental medicine. While increased dye retention cannot be definitely correlated with specific disturbance of the various functions of the liver, it does indicate liver damage of some sort, the degree of dye retention giving a grossly quantitative estimation of the extent of the damage.

Although the standard bromsulphalein test is applicable to the larger laboratory animal, such as the dog or the rabbit, the relatively large quantities of blood required for the test prevent its use in small laboratory animals which in many experiments are those of choice. We have, therefore, modified the method for application to rats and mice. The technic is simple, rapid, and in the rat it can be repeated at daily intervals by using alternate saphenous veins.

The application of the test to small animals is made possible by the replacement of the customary pipettes and test tubes with glass-capillaries, a suggestion made to us by Dr. A. N. Richards. The pyrex glass capillaries are machine drawn to an outside diameter of 1.2 mm and an inside diameter of 0.9 mm and are so uniform that minute fluid volumes can be estimated quite accurately by measuring the length of the fluid column.

Experimental Technic. The rat is weighed, securely tied to a board, and 10 mg per kg of a 1.0% bromsulphalein solution is injected into the saphenous vein. Four minutes and 50 seconds following the injection, the tail, having just been dipped into warm water and dried, is clipped and two capillaries 9 cm in length are filled with blood, leaving about 2 cm free for sealing. The ends of the capillaries are flame-sealed and the tubes are immediately spun at 3000 rpm; usually 2 to 3 minutes suffice to separate the serum and cells. Breakage of the pyrex capillaries in the centrifuge at 3000 rpm is rare in our experience, but they do not withstand much higher speed.

1 Rosenthal, S. N., and White, E. C., *J. Pharm. and Exp. Therap.*, 1924, **24**, 365.

2 Rosenthal, S. N., and White, E. C., *J. A. M. A.*, 1925, **84**, 112.

The serum containing portion of the first capillary is broken off and serum in excess of 20 mm of capillary length (approximately 0.014 cc) is expelled. A 10% solution of sodium hydroxide is drawn up into the capillary to a distance of 1 mm (approximately 0.0007 cc) and then both serum and hydroxide are blown onto a glass slide and thoroughly mixed. This solution is then drawn back into the capillary and is ready for direct comparison with the bromsulphalein standard. In making the reading the non-alkalized serum of the second capillary is placed behind the standard in a suitable comparator box.

The standards are made on the assumption that the blood volume of the rat is approximately 10% of the body weight. In setting up the standard a 1-20 dilution with alkali is made so that the alkali dilution occurring in the test is corrected for. The 100% standard is obtained by adding 1 cc of a 5% solution of bromsulphalein and 12½ cc of 10% sodium hydroxide to 249 cc of water. By appropriate dilutions a series of standards from 5 to 90% is prepared, drawn into capillaries and sealed. These standards, if protected from bright light will keep for several months.

In a series of 70 normal rats, the dye retention at the end of 5 minutes was found to be 10% or less in all but 5 of the animals. Two of the latter showed a 20% retention and the remaining 3 showed a 15% retention.

That the percentage retention readings can be duplicated on the same animal and that the dye in itself does not damage the liver is shown by the following experiment: Ten normal rats, all of whom showed 10% retention or less were given 10 mg per kg of bromsulphalein 7 times over a period of 2 weeks. At the end of this period all the animals still showed the same degree of dye retention as at the beginning of the experiment.

This test can be applied to mice by the same technic as described above for the rat. It is necessary, however, to give the mouse 25 mg of bromsulphalein per kg and the color standards have to be made up to conform with this dosage. Either the saphenous or the tail veins may be used for injection of the dye. The test is not as satisfactory in the mouse as it is in the rat as on occasion difficulty is experienced in separating the serum from the cells. Furthermore, the number of tests on an individual mouse is usually limited as the veins tend to thrombose following the injections.

In order to correlate the dye retention with the severity of liver damage, rats were subjected to various degrees of phosphorus poisoning. Table I gives the degree of dye retention in these poisoned

TABLE I.
Bromsulphalein Retention Following Phosphorous Poisoning.
10% dye retention before adminis. of P.

Rat No.	Daily s.c. dose of P in sesame oil mg/100 g	No. of days	% dye retention after administr. of P	Liver damage estimated by histological study
1	.375	2	15	0
2	.375	2	10	0
3	.375	4	50	+++
4	.375	4	50	+++
5	.375	5	60	++++
6	.375	5	45	+++
7	.75	2	20	0
8	.75	2	15	0
9	.75	3	40	++
10	.75	3	20	0
11	.75	4	45	++
12	.75	4	60	++++

animals together with the amount of liver damage as judged by study of microscopic sections stained with hematoxylin-eosin, and Sudan IV. These data show a relation in the amount of phosphorus given to the degree of dye retention and to the liver damage as estimated histologically.

Summary. A simple and rapid technic for performing the Rosenthal-White bromsulphalein liver function test on rats and mice is described. The test gives a roughly quantitative estimate of the degree of liver damage and since it can be repeated on the same animal it is of use in following the progress or regression of liver disturbances.

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Current Control in Electroshock Therapy.*

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Electroshock treatment consists in the passage of alternating current for a few tenths of a second through electrodes placed on the temples of the patient. The intensities of the current vary from 200 milliamperes to the neighborhood of 1,000 milliamperes. The customary procedure is to apply a certain voltage obtained from the

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