The Significance of Dosage and Time Factors on the Value of the Bromsulphthalein Test for Liver Function*

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THE technique originally described by Rosenthal and White (1) for testing the excretory function of the liver by means of an intravenously injected dose of bromsulphthalein has been modified so often that one frequently is at a loss to know what particular method to employ and also how to interpret the results from the various procedures. In an attempt to bring about a standardization of the technique and consequently greater confidence in it, we have compared in a selected group of subjects, including normals and some with hepatic disease, several of the suggested modifications. We have found that the collection of a single blood specimen 30 minutes after the intravenous injection of 5 mg. of the dye per kilogram of body weight constitutes an adequate test for ordinary clinical purposes.

PROPOSED METHODS

The original technique consists in estimating, by comparison with suitable standards, the amount of dye retained in the blood stream at 5 and 30 minute intervals after the injection of 2 mg. of bromsulphthalein (in a 5 per cent solution) per kilogram of body weight. After securing a sample of blood from a vein in one arm, the dye is injected and then the two subsequent specimens at the proper time intervals are collected from the other arm for estimation of the contained dye. Normally, from 20 to 50 per cent of the dye is stated to be present in the circulating blood after 5 minutes, while all of it should have disappeared after 30 minutes.

The following modifications of this technique have been described. O'Leary et al (2) (1932) introduced the use of a test dose of 5 mg. per kilogram of body weight. In the series of subjects whom they regarded as controls, a retention of 0 to 8 per cent of the injected dye at the end of 30 minutes or of 0 to 4 per cent at the end of 60 minutes was accepted as normal. Shay and Schloss (3), Robertson et al (4) and Soffer (5) expressed the opinion that the 5 mg. dose was more sensitive and gave a higher percentage of positive results than the 2 mg. one. None of these investigators, however, compared the two methods in the same subject. Magath (6) used the 5 mg. dose and withdrew only one sample of blood 60 minutes after injection of the dye; he regarded a retention of more than 6 per cent as abnormal. Israel and Reinhold (7) employed the same dose, but measured the dye concentration 30 minutes after injection. A retention of more than 6 per cent was considered abnormal. Rosenberg and Soskin (8) and Cates (9) also used the 5 mg. dose but accepted a retention of 0 to 10 per cent at the end of 30 minutes as within normal limits. Thus in the various reports, one finds a variation in the amount of dye injected, in the time intervals after which samples of blood are withdrawn and in the values which have been accepted as representing the normal. In an attempt to evaluate these factors we have compared the results when 2 and when a 5 mg. dose was administered in the same subject; also, the relative merits of taking the blood sample at 30 or at 60 minutes after the injection of the dye also were investigated.

Various other modifications of the test, which we have disregarded, have been proposed. MacDonald (10) made a rather extensive study of the rate of disappearance of the dye from the blood after injecting amounts varying from 2 to 10 mg. per kilogram of body weight. He withdrew specimens of blood every 5 minutes and plotted a curve of the rate of disappearance of the dye. Using the 2 mg. dose, he found the blood stream in the normal subject to be dye-free after 18 minutes, and, with the 5 mg. dose, after 25 minutes. White and his associates (11) retained the use of the 2 mg. dose, but withdrew 3 samples of blood within 15 minutes. They regarded as normal in adults a retention of 80 per cent at the end of 2, of 15 per cent at the end of 5, and of 5 per cent at the end of 15 minutes. Such methods, however, require considerable equipment and time and also are more or less disturbing to the patient. Furthermore, specimens of blood taken before one-half hour are apt to give dye readings too variable for proper interpretation (Shay and Schloss (3)), and the mixing of an injected dye with the blood stream is considered to be incomplete in less than 20 minutes (Sunderman and Austin (12.)) Consequently those modifications in which blood samples are withdrawn sooner than 30 minutes after injection have not been included in our comparisons.

SUBJECTS AND PROCEDURE

A group of 50 adult patients with significantly enlarged livers or with a primary disease in which hepatic function was believed to be disturbed were selected from admissions to the medical wards of this hospital. The only other requisite was that the direct van den Bergh reaction of the serum be either negative or delayed, since a retention of the dye practically always occurs in patients whose serum gives either a

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biphasic or an immediate direct reaction. The tests, however, were performed in a series of 17 patients with a positive direct van den Bergh reaction, merely to obtain data to substantiate this point. In addition, twelve patients in whom no suspicion of hepatic disease had arisen were used as controls.

The tests were performed on successive days with the subjects fasting for 12 hours before each of them. Dye was injected intravenously in amounts of 2 and later of 5 mg. per kilogram of body weight and blood samples were taken at 30 and at 60 minute intervals after each injection. The same set of standards (Hynson, Westcott and Dunning, Baltimore), prepared according to Rosenthal's (1) recommendations for the 2 mg. dose, was used for each determination. When employed in estimating the retention of dye in the serum after the 5 mg. dose, the reading was multiplied by 2/5. All the estimations were made by the same person. The direct van den Bergh reaction was determined in each instance prior to the dye tests, and the degree of bilirubinemia was determined either by the indirect van den Bergh reaction or by the icterus index.

RESULTS

Thirty-eight of the 50 hepatic cases, on receiving the 5 mg. per kilogram dose, showed some retention of the dye after 30 minutes, and 27 of these even after 60 minutes. On receiving the 2 mg. dose, 21 of the 50 showed retention after 30 minutes, and only 3 of them after 60 minutes (Table I.)

Of the additional 17 patients, who gave either an immediate direct or a biphasic van den Bergh re-

TABLE I

The retention of dye in the serum of patients 30 and 60 minutes after injection of 2 and 5 mg. doses. The direct van den Bergh reaction in all cases is "negative or delayed"

Case No.	Percentage Retention of Dye				Van den Bergh	
	5 mg. per Kg.		2 mg. per Kg.			
	30 Min.	60 Min.	30 Min.	60 Min.	Direct	Indirect in mg. Per Cent
1	0	0	0	0	negative	±0.1
2	0	0	0	0	"	±0.1
3	0	0	0	0	"	±0.1
4	0	0	0	0	"	±0.1
5	0	0	0	0	"	±0.1
6	0	0	0	0	delayed	0.3
7	0	0	0	0	"	0.35
8	0	0	0	0	"	0.4
9	0	0	0	0	"	0.4
10	0	0	0	0	"	0.65
11	0	0	0	0	"	0.8
12	0	0	0	0	"	0.95
13	4	0	0	0	negative	±0.1
14	4	0	0	0	"	±0.1
15	4	0	0	0	delayed	±0.1
16	4	0	0	0	"	±0.1
17	4	0	0	0	negative	±0.1
18	4	0	0	0	"	0.4
19	8	0	0	0	"	±0.1
20	12	0	0	0	"	±0.1
21	12	0	0	0	delayed	0.5
22	12	4	0	0	"	0.5
23	12	6	0	0	negative	±0.1
24	12	4	0	0	delayed	1.2
25	12	6	0	0	"	0.5
26	16	12	0	0	negative	0.4
27	20	0	0	0	delayed	0.75
28	20	5	0	0	"	±0.1
29	28	8	0	0	"	0.3
30	8	4	5	0	negative	±0.1
31	12	0	5	0	delayed	0.5
32	12	4	5	0	negative	0.5
33	16	4	5	0	"	±0.1
34	16	8	5	0	"	±0.1
35	20	8	5	0	"	0.5
36	32	16	5	0	delayed	±0.1
37	34	28	5	0	"	±0.1
38	16	12	10	0	"	0.5
39	20	4	10	0	"	0.6
40	20	8	10	0	negative	±0.1
41	24	8	10	0	"	±0.1
42	24	8	10	0	"	±0.1
43	24	12	10	0	"	±0.1
44	28	8	10	0	delayed	0.9
45	28	10	10	0	negative	0.95
46	36	8	10	0	"	±0.1
47	36	12	10	0	delayed	1.6
48	36	24	10	5	"	0.25
49	40	12	20	5	"	1.4
50	64	32	30	10	"	0.3

TABLE II

The retention of dye in patients in whom the direct van den Bergh reaction was "biphasic" or "immediate"

Case No.	Van den Bergh		Icteric Index	Percentage Retention of Dye			
				5 mg. per Kg.		2 mg. per Kg.	
	Direct	Indirect in mg. Per Cent		30 Min.	60 Min.	30 Min.	60 Min.
1	Biphasic		10	0	0	0	0
2	"		12	40	0	0	0
3	"		12	12	6	0	0
4	"		10	40	20	10	0
5	"		16	36	16	20	5
6	"		12	45	30	30	10
7	Immediate	0.5		16	8	0	0
8	"	0.5		24	0	10	0
9	"	0.5		48	28	35	10
10	"	0.7		40	32	20	0
11	"	0.85		36	4	20	0
12	"	1.0		20	5	15	0
13	"	1.1		50	36	5	0
14	"	1.9		64	40	30	10
15	"	2.9		90	60	80	40
16	"	3.7		70	40	50	30
17	"	4.8		64	44	40	20

action, 16 retained some of the dye for 30 minutes, and 14 for 60 minutes after the 5 mg. dose; while 13 showed retention for 30 minutes, and 7 for 60 minutes after the 2 mg. dose (Table II.)

None of the 12 controls showed retention at the 30 minute period, after either the 2 or the 5 mg. test dose.

COMMENT

These results indicate that a dose of 5 mg. of bromsulphthalein per kilogram of body weight and a single specimen of blood withdrawn after 30 minutes, as employed by Israel and Reinhold, offer a more satisfactory test than does the use of the smaller dosage or a longer time interval. It is simple to perform, requires little equipment and subjects the patient to only two venipunctures. It gives a sensitive and relatively reliable estimation of hepatic excretory ability. These results indicate that the 5 mg. dose is more sensitive in showing impaired hepatic function than the 2 mg. one, and that some patients, presumably with impaired hepatic excretion, are able to clear their blood stream completely of the dye within 60 minutes, even when the 5 mg. dose is employed.

That the use of the 5 mg. dose of the dye does not overtax the excretory ability of a supposedly normal liver is evidenced by the absence of its retention in our series of controls, as well as by the observations of MacDonald (10) in his normal subjects. Furthermore, 24 per cent of our cases, suspected of having hepatic disease, had no dye retention at the end of 30 minutes. Thus any dye retention 30 minutes after the injection of a dose of 5 mg. of bromsulphthalein per kilogram of body weight may be considered as evidence of impaired hepatic excretory ability.

In the subjects with a biphasic or an immediate direct van den Bergh reaction, the dye was retained as expected. This type of van den Bergh reaction, as a matter of fact, in itself indicates impaired hepatic excretory ability, and in this respect at least the dye test is unnecessary. In such a patient, however, the dye test may furnish base-line information from which improvement may be judged after the jaundice has subsided.

CONCLUSIONS

1. The employment of a dose of 5 mg. of bromsulphthalein per kilogram of body weight yields a higher percentage of positive results than does that of a dose of 2 mg., and at the same time the larger dose does not overtax the excretory function of the liver.

2. The blood secured 30 minutes after the injection of the 5 mg. dose contains the dye more frequently than that secured after 60 minutes, and the 60-minute specimen adds nothing to the diagnostic value of the test.

3. The retention of any of the dye in the blood 30 minutes after the injection of a 5 mg. dose should be regarded as an indication of impaired hepatic excretion.

4. The technique, as used by Israel and Reinhold, that requires a 5 mg. dose of the dye and a single specimen of blood, secured after 30 minutes, may be regarded as the most satisfactory of the suggested modifications of the bromsulphthalein test.

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