but also with all the findings in this connection that we have examined; for example, to name only a few, the findings of Rosenblueth,[3] that there are two substances produced by the action of "sympathin" on receptive substances; the findings of Brown and Feldberg,[4] that potassium ions stimulate the curarized sympathetic ganglion which is not acted upon by acetylcholine; and the findings of Lánczos,[5] that calcium ions in addition to "sympathin" are liberated by stimulation of the sympathetic fibers to the frog's heart, and that these same calcium ions in the strength (0.01%) found in Ringer solution produced a marked action in the ergotaminized heart.

Further details, other drug actions and the histological studies will be presented in a following paper. We wish to thank Merck & Co. for the carbaminoyl choline.

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Bromsulphalein Dye Retention Test as a Measure of Functional Activity of Reticulo-Endothelial System.

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In the course of studies of the reticulo-endothelial system, it became apparent that some method of measurement of the function or degree of functional impairment obtained with the present so-called blockading methods, would be of great value in interpreting the experimental results. Of the various methods which offered themselves as tests, a dye excretion or clearance test, which depended upon the integrity of the reticulo-endothelial system seemed most suitable for adaptation to our needs.

Various dyes have been suggested as being selectively removed from the blood stream by the reticulo-endothelial system. Landsberger[1] reported using Congo Red for this purpose, but obtained inconstant results. Merklan and Wolf,[2] Saxl and Donath,[3] Shellong

[3] Rosenblueth, A., *Am. J. Physiol.*, 1932, **101**, 149; **102**, 12.

[4] Brown, G. L., and Feldberg, W., *J. Physiol.*, 1935, **86**, 10 P.

[5] Lánczos, Anna, *A. f. Path. u. Pharm.*, 1936, **180**, 312.

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[1] Landsberger, J., *Z. f. Immunitatsforsch.*, 1930, **65**, 385.

[2] Merklan and Wolf, *Bull. et Mem. d. l. Soc. Med. des Hosp.*, 1925, **49**, 1180.

and Eisler,[4] and others have reported that phenoltetrachlorphthalein is removed from the blood stream by the reticulo-endothelial system. Herlitz[5] suggested that bromsulphalein might be removed from the blood by the macrophage system.

Klein and Levinson,[6] using bromsulphalein, found that they could obtain definite retention of bromsulphalein following splenectomy, and rather more marked retention after injections of India ink in dogs. The availability of this dye, as well as its freedom from toxic manifestations led us to consider it as a measure of the functional impairment of the reticulo-endothelial system obtained with India ink and other "blockading" agents.

Bromsulphalein tests were run upon 24 normal dogs to obtain the normal values for dye clearance in the dog. Two mg. of dye per kilo body weight was injected intravenously, and blood samples drawn at 5 and 30 minutes. The blood was oxalated and centrifuged to obtain the plasma. The dye was visualized by adding 2 drops of N 10 NaOH. If the materials injected for blockade obscured the reading, precipitation with acetone was used before alkalinizing. This technique has been previously described by us.[7]

The results of the normal tests were constant for this series and no deviation in normal animals subsequently tested has been found. Values of 5% to 15% dye in the first sample and complete absence of dye in the second sample were found. No variations in dye excretion have been seen during short periods of ether or barbital anesthesia.

In the first series of experiments we were interested in confirming the observations of Klein and Levinson, that splenectomy, which may be assumed to cause little or no demonstrable liver damage and at the same time to remove a recognizable fraction of the reticulo-endothelial system, causes retention of bromsulphalein, and that retention of the dye also follows injections of India ink.

Three dogs were anesthetized with ether, and subjected to splenectomy. Tests were run immediately following the removal of the organ. Test readings of 20% dye in the 5 minute sample and from 5 to 10% in the 30 minute sample were obtained in all animals. This is a definite though slight retention of the dye. In sections of the liver of 2 of the dogs fixed immediately following the test, no

[3] Saxl, P., and Donath, F., *Wien. Arch. f. inn. Med.*, 1926, **13**, 7.

[4] Shellong, F., and Eisler, B., *Z. f. d. ges. exp. Med.*, 1928, **58**, 738.

[5] Herlitz, C., *Acta Pediatrica*, 1931, **12**, Sup. 5, 1.

[6] Klein, R., and Levinson, S. A., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 179.

[7] Dragstedt, C. A., and Mills, M. A., *J. Lab. Clin. Med.* (in print).

demonstrable changes in the organ could be found. The third dog was tested daily following the operation. The dye clearance was again within the normal range in 48 hours.

In experiments on the reticulo-endothelial system, it has been assumed that multiple injections of blockading materials produce a progressively increasing blockade of the system until a maximum, or theoretical "complete" blockade is reached, and that these injections must be continued to maintain the blockade. Rapid recovery of function is known to follow discontinuance of the injections. Accordingly, dye tests were run following single and multiple injections of India ink and saccharated iron oxide, as recognized blockading materials. Two hours following a single injection of 40 cc. of 8% India ink, dye retentions of 25% in the first sample and 15% in the second sample were obtained. Twenty-four hours later the readings were 20% and 5% respectively. Tests run 24 hours after each of a series of daily injections of 20 cc. of 8% India ink showed a progressively increasing retention of dye with a maximum after the sixth injection of 60% and 40% in the 2 samples. Readings of 20% and 5% were found 96 hours following the last injection. The animals were not followed longer than 96 hours after stopping the injections.

Repeating these procedures with injections of 15 cc. of 20% saccharated iron oxide, less marked results were obtained. A single injection of the material produced only slight dye retention after 2 hours with normal values after 24 hours. Multiple injections of iron oxide caused only a slightly increasing dye retention although readings of 20% and 5% were obtained after 2 injections. The dye clearance was again normal after 96 hours.

In several types of acute experiments on the reticulo-endothelial system it appeared that if a rapid method of blockade could be used it would eliminate much of the labor and hazard of the multiple injection method. To this end a modified Woodyatt pump which would deliver a continuous flow of 2 cc. per minute of the blockading material, (India ink, 8%, and iron oxide, 5% or 10%), was used. Average total amounts of 150 to 250 cc. of material were injected for blockade. Bromsulphalein test readings taken at intervals of 5 minutes to 3 hours following such injections gave average readings of 60% to 80% in the 5 minute sample, and 50% in the 30 minute sample for India ink. With iron oxide, values of 40% (in one instance 60%) were obtained in the first sample and 10% to 30% in the 30 minute sample.

Summary. The observations of Klein and Levinson, that the

intravenous injection of India ink in dogs causes retention of bromsulphalein are confirmed. The dye retention becomes progressively greater with repeated daily injections. These observations, in conjunction with the finding that splenectomy produces a period of temporary dye retention may be interpreted as indicating that bromsulphalein excretion is related in some way to the reticulo-endothelial system, and that the degree of dye retention may be used as a quantitative expression of the degree of "blockade" or impairment of the reticulo-endothelial system produced by blockading procedures. A marked degree of impairment, as measured by the dye retention can be produced during the course of 2 or 3 hours by a continuous injection of blockading agents. Saccharated iron oxide was found to be less effective as a blockading agent than India ink as measured by the bromsulphalein dye retention which it produces.

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Anaphylaxis in the Normal Unanesthetized Dog.

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During the course of an investigation carried out in this laboratory, anaphylaxis was induced in 61 normal unanesthetized dogs. Since references in the literature to anaphylaxis produced under these conditions in a large series of dogs are meager, it was deemed advisable to record our results.

The dogs were sensitized by injecting 5 cc. of normal horse serum subcutaneously and 5 cc. intravenously. After a 16 day incubation period the animals were given the provocative dose of horse serum consisting of 10 cc. administered intravenously.

Evaluation of the anaphylactic reaction in unanesthetized animals depends on the observation of symptoms and not on the determination of measurable responses; so it is subject to individual interpretation. This difficulty was overcome as far as possible by dividing the reactions into several classes according to their intensity, and then arbitrarily designating certain sets of symptoms as characteristic of each class. Thus all reactions are described as either slight, moderate, moderately severe, or severe. In a slight reaction the animal shows only slight transitory symptoms, little or no dis-