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Moore A. Mills; ... et. al

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THE RETICULO-ENDOTHELIAL SYSTEM AND ANAPHYLAXIS IN THE DOG

MOORE A. MILLS[1] AND CARL A. DRAGSTEDT

WITH THE ASSISTANCE OF FRANKLIN B. MEAD

From the Department of Pathology and the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Illinois

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The reticulo-endothelial system has been implicated in the majority of immunologic reactions. There have been numerous studies of the relationship of this system of cells to the anaphylactic reaction, particularly in the rabbit and guinea pig. The results of these studies are in many instances conflicting and irregular. The reader is referred to the review by Hill and Martin (1) for a résumé of these reports. We have found only one reference (2) to the use of the dog in this connection. We (3, 4) have found the dog to be a highly satisfactory animal in which to record objectively and with precision the varying degrees of severity of anaphylactic shock. The principal experimental approach to the study of problems involving the macrophagic system has been by means of the so-called blockade with colloidal material under the assumption that the cells, laden with the extraneous material, were rendered either functionless or their function was so seriously impaired that demonstrable alterations would occur in the reactions in which they were involved. We have elsewhere reported (5) that in the dog there is evidence to indicate that the degree of blockade of the reticulo-endothelial system can be more or less quantitatively correlated with bromsulphalein dye-retention. Because of the ability to record the anaphylactic reaction in a precise way and also to state the degree of blockade in somewhat quantitative terms it was considered that the dog might be

[1] Frederick Fobert Zeit Fellow.

a highly suitable animal in which to determine the relationship of the reticulo-endothelial system to anaphylaxis.

EXPERIMENTAL

Healthy adult dogs from 6 to 16 kgm. in weight were sensitized to horse-serum by the simultaneous injection of 5 cc. intravenously and 5 cc. subcutaneously. In a large series of animals this has proven to be a highly successful sensitizing technic. After an incubationary period of 16 to 41 days the animals were anesthetized with ether and barbital, the carotid was cannulated for a blood-pressure tracing and the femoral vein exposed. The test-dose (10 cc.) of horse-serum was injected into the femoral vein and the severity of shock noted on the tracing and expressed according to arbitrary criteria as follows: $++++$ = a reaction fatal within 30 minutes, $+++$ = a severe reaction with blood-pressure at shock-level for 30 minutes, $++$ = a moderately severe reaction with fall of blood-pressure to shock-level but with partial or complete recovery within 30 minutes, $+$ = a mild reaction with a fall in blood-pressure that does not reach shock-level and is rather promptly recovered from, and 0 = no definite objective signs of shock. This classification of reactions in dogs has proven useful in other studies previously reported.

Prior to introduction of the test-dose of serum, the dogs were "blockaded" by the intravenous administration of either Indian ink or saccharated iron oxide. This was done either "intermittently" by repeated daily injections of 15 to 20 cc. of the solutions or "continuously" by injecting from 150 to 325 cc. during the course of 2 to 3 hours on the day of the experiment. The solutions of ink were freshly prepared by boiling filtered ink in a waterbath for 30 minutes and diluting to the desired concentration with sterile saline solution. The saccharated iron oxide solutions were freshly prepared with sterile distilled water. The number of injections, concentrations of solutions, etc., are indicated in the table. Just prior to the test-dose of serum, a bromsulphalein dye-retention test was performed, using 2 mgm. of the dye per kilogram of body-weight and determining the concentration of dye in samples of plasma drawn after 5 and after 30 min-

utes. It is assumed that this dye-retention expresses the degree of blockade achieved at the time of the experiment (5). The essential results are shown in table 1.

TABLE 1

Reticulo-endothelial blockade and anaphylactic shock in dogs

EXPERIMENT	BLOCKADING AGENT	METHOD OF BLOCKADE	BROMSULPHALEIN RETENTION PRIOR TO SHOCKING DOSE		DEGREE OF ANAPHYLACTIC SHOCK
			5 minutes	30 minutes	
			per cent	*per cent*	
142	Indian ink, 8–30%	5 daily intravenous injections of 20 cc.	60	20	++++
143	Indian ink, 8–50%	7 daily intravenous injections of 20 cc.			++
144	Indian ink, 8–50%	7 daily intravenous injections of 20 cc.			++++
145	Indian ink, 8–50%	8 daily intravenous injections of 20 cc.	40	20	++
146	Indian ink, 8%	252 cc. on day of shock	80	60	+++
147	Iron oxide, 5%	263 cc. on day of shock	30	10	0
148	Iron oxide, 5%	325 cc. on day of shock	40	30	+++
169	Iron oxide, 5%	265 cc. on day of shock	40	10	++++
175	Iron oxide, 5%	300 cc. on day of shock	60	30	+?
178	Iron oxide, 5%	260 cc. on day of shock	60	30	++++
180	Iron oxide, 5%	300 cc. on day of shock	30	10	+++
188	Iron oxide, 20%	4 daily injections of 15 cc.	10	0	++
189	Iron oxide, 20%	4 daily injections of 15 cc.	15	0	++++
190	Indian ink, 8%	200 cc. on day of shock	60	40	0
192	Indian ink, 8%	150 cc. on day of shock	80	50	++++
195	Indian ink, 8%	160 cc. on day of shock	80	50	+++
196	Indian ink, 8%	8 daily injections of 20 cc. (5 of these before sensitizing dose)	20	5	++++
197	Iron oxide, 20%	8 daily injections of 15 cc. (5 of these before sensitizing dose)	10	0	++

Explanatory: All dogs sensitized to horse-serum. Incubationary period 16 to 41 days in all except experiment 190 which was 102 days. Test-dose, 10 cc. of horse-serum via femoral vein.

SUMMARY AND DISCUSSION

In 18 dogs sensitized to horse-serum two different blockading agents (Indian ink and saccharated iron oxide) were administered

by various methods. Moderately severe to fatal reactions were produced in 15, a questionable reaction in one, and no evidence of shock in 2. Of these latter the incubationary period of 102 days in experiment 190 is so long as to raise doubt concerning the validity of the experiment. The percentile distribution of the varying degrees of shock in 59 control animals was as follows: $++++ = 28$, $+++ = 13$, $++ = 48$, $+ = 8$, $0 = 3$. The corresponding percentages in the experimental series are respectively 38, 22, 22, 5 and 11. There is a slight shift in the direction of increased severity of reaction, but we do not believe the difference is great enough to be significant. There is thus no indication from these experiments as a whole that reticulo-endothelial blockade in any way modifies the reaction resulting in anaphylactic shock. Use of the bromsulphalein dye-retention test as a measure of the degree of blockade produced by the various agents and methods of administration gave no indication of any relationship between the degree of blockade and the severity of the shock. Fatal reactions occurred as frequently in the animals with marked retention of dye as in those with comparatively little retention. In 2 experiments the animals were blockaded prior to the administration of the sensitizing injections with no apparent effect upon the sensitization as judged by the subsequent shocks. We are thus unable to confirm the report of Petersen, Jaffe, Levinson and Hughes that reticulo-endothelial blockade diminishes the anaphylactic reaction in dogs. These workers used only 5 animals, the antigen used was egg-white which has been reported to be anaphylactogenically inferior to horse-serum for the dog (6), and the criterion used for measuring shock was a chemical study of lymph from the thoracic duct. No direct comparison of results is therefore possible. With regard to the relatively extensive literature on anaphylaxis and reticulo-endothelial blockade in other laboratory animals there are contradictory reports (1). The methods of blockade have varied with respect to the routes of administration (e.g., subcutaneous, intraabdominal, and intravenous), different blockading agents have been used, and the shocking dose of antigen has been administered subcutaneously, intraabdominally or intravenously. It is therefore difficult to

compare most of these reports. It has, however, occurred to us that in many experiments in which the shocking dose of antigen has been given other than intravenously, that the reported protection against anaphylactic shock has been possibly due to a reduced or delayed absorption of the antigen rather than to a specific effect upon the anaphylactic reaction. In some of our experiments on rabbits and guinea pigs this has seemed to be the case. In 7 horse-serum sensitized guinea pigs in which Indian ink was injected intraabdominally on the last 2 days of the incubationary period, the intraabdominal injection of horse-serum provoked no anaphylactic symptoms, while all of the 7 controls showed definite symptoms and 2 had fatal reactions. In 8 horse-serum sensitized rabbits that had received intraabdominal injections of Indian ink, the intraabdominal test injection of horse-serum was followed by shock in 2. Of the remaining six, 4 showed definite symptoms when subsequently injected intravenously. In 7 control horse-serum sensitized rabbits, the intraabdominal injection was followed by symptoms of shock in 5. Of the remaining 2, one showed symptoms following intravenous injection. These experiments are not sufficiently numerous to warrant final conclusions but we believe that the simplest explanation of these results is that the ink in these experiments in some way interfered with the absorption of the antigen from the peritoneal cavity.

CONCLUSIONS

Reticulo-endothelial blockade has no apparent effect upon the anaphylactic reaction in horse-serum sensitized dogs. It does not prevent the reaction of the test dose of serum nor in 2 experiments was there any evidence that it prevents sensitization by the initial injections.

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