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## Isohemolysins and Isoagglutinins Occurring in Dogs.

ANGUS WRIGHT. (Introduced by G. H. Whipple.)

*From the Department of Pathology, The University of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

*Plasmapheresis* as used in this laboratory means the removal of blood cells and plasma by bleeding followed at once by the replacement of normal red cells suspended in a modified Locke's solution. In this way plasma depletion is readily accomplished and the regeneration of new plasma protein as influenced by various factors can be studied.[1] In the course of such experiments many unfortunate developments may complicate the picture and obscure results. Among these complications *hemolysis* has been noted all too frequently in certain dogs following exchanges. These dogs became sick with vomiting, diarrhœa, hemoglobinuria, jaundice, anemia and even severe shock. Other dogs, however, have received similar exchanges of red cells from 3 to 6 times a week for long periods of time from the same group of 11 donor dogs without untoward effect.

It is known that there are indistinct differences of blood types in dogs.[2] The rarity of occurrence of these differences and difficulty in their demonstration have made consideration of blood grouping in dogs unnecessary for ordinary experimental procedures. Isohemolysins and isoagglutinins in dogs following repeated direct transfusions have been reported.[3] Recently, Melnick, Burack and Cowgill[4] have reported incompatibilities occurring in dogs on plasmapheresis experiments, similar to those noted in this laboratory. Baldridge[5] noted similar changes resulting in the death of one dog which had previously received several transfusions from an originally compatible donor.

Determination of the results following the *in vitro* mixture of the sera of the various recipient dogs with the red cells of the

[1] McNaught, J. B., Scott, V. C., Woods, F. M., and Whipple, G. H., *J. Exp. Med.*, 1936, **63**, 277.

[2] Wiener, A. S., *Blood Groups and Blood Transfusion*, Springfield, Ill., Charles C. Thomas, 1935.

[3] Ottenberg, R., Kaliski, D. J., and Friedman, S. S., *J. Med. Res.*, 1913, **28**, 141.

[4] Melnick, D., Burack, E., and Cowgill, G. R., PROC. SOC. EXP. BIOL. AND MED., 1936, **33**, 616.

[5] Baldridge, C. W., *Arch. Int. Med.*, 1934, **54**, 517.

donor group were made. Observations were made of both macroscopic and microscopic hemolysis and agglutination.

The procedure for the macroscopic method consisted of mixing in a test tube (7 mm. inner diameter) 0.25 cc. of the clear unhemolyzed recipient serum with 0.5 cc. of a 5% suspension of fresh, washed red cells from the donor to be tested. The mixture was then incubated at 37°C. for 2 hours with observation at half hour intervals. Hemolysis was indicated by change of the cloudy cell suspension to a clear cherry-red color. Agglutination occurred early as indicated by marked clumping of the red cells at the bottom of the tube in distinct contrast to the controls. The microscopic tests were made by use of hanging drop preparations.

The first group of dogs consists of 3 animals which were used in plasmapheresis experiments until development of the abnormalities described was so severe as to preclude their further use.

*Dog 34-174,* a female mongrel hound, was used in a plasmapheresis experiment for a period of over one month, and received in this time 22 red cell exchanges. The animal then developed marked hemolysis of the red cells, became jaundiced, was weak, feverish and refused food following injection of red cells. The experiment was then terminated and the dog was not used for a period of 8 months. The dog's serum was then tested *in vitro* against the red cells of the group of 11 donor dogs. The red cells of 5 of the donor group were hemolyzed and agglutinated while the remaining 6 were compatible. The dog was then given intravenously, without reaction, 15 cc. of washed, packed red cells, suspended in an equal quantity of Locke's solution, from a compatible donor dog. Two days later, an injection of 5 cc. of similarly prepared red cells from another compatible donor was given, and on the next day, 6 cc. from another compatible donor. There was no evidence of reaction following any of these injections. After this the red cells of 2 of these donors, previously compatible by both *in vitro* and *in vivo* test, were found to be incompatible *in vitro* as evidenced by hemolysis and agglutination.

*Dog 34-180,* a female mongrel terrier, first developed evidence of hemolysis of red cells after it had received 4 red cell exchanges in a period of 10 days. With following injections of red cells there was increased hemolysis, hemoglobinuria, diarrhœa. In all, 12 exchanges of red cells were made over a period of 20 days when the experiment was terminated. After a period of 8 months' rest the serum of this dog was tested *in vitro* with the red cells of the group of 11 donor dogs. The red cells of 7 of the donors were

hemolyzed and agglutinated while the red cells of the remaining 4 showed no evidence of incompatibility.

*Dog 34-53,* a mongrel male poodle, was used in plasmapheresis experiments for a period of over one month, and received 31 red cell exchanges before similar abnormalities were noted and the experiment was terminated. After a period of 9 months, this dog was given 3 red cell exchanges. Following each injection, the dog developed hemolysis of red cells, hemoglobinuria, vomiting and diarrhœa. After a period of 3 more months this dog was given several intravenous injections of red cells from donors which were incompatible by *in vitro* test. Severe reactions marked by the signs described previously immediately followed each injection. The dog was then given without reaction, intravenous injections of red cells from 2 dogs compatible by *in vitro* test. Several days later, the red cells of these 2 previously compatible dogs were markedly incompatible as indicated *in vitro* by hemolysis and agglutination. At this time, the serum of this dog tested against the red cells of the donor group, rapidly hemolyzed and agglutinated the red cells of 7 of these dogs while 4 were compatible.

The red cells of the above 3 plasmapheresis recipient dogs, tested *in vitro* with the sera of the group of donor dogs, showed no evidence of incompatibility.

In contrast to the difficulties experienced with the 3 dogs discussed above, many plasmapheresis experiments have been performed in this laboratory in recent years without development of such abnormalities in the recipient dogs. The following 2 dogs illustrate the frequently repeated, successful use of dogs in plasmapheresis. *Dog 33-11,* a female mongrel hound, was used for a period of over 5 months, during which time it received 110 red cell exchanges from the same donor group without evidence of reaction. *Dog 34-152,* a male mongrel hound, was used for a period of over 9 months and received 190 red cell exchanges without reaction.

The sera of these 2 animals showed no evidence of incompatibility with the red cells of the donor group in hanging drop preparations. However, in the macroscopic procedure, mixture of the sera of these 2 animals with the red cells of the donor dogs, showed hemolysis which was not evident in controls with the sera of normal dogs. In these observations there was no evidence of macroscopic agglutination, nor was hemolysis as marked as when such tests were made with the sera of the 3 plasmapheresis dogs first discussed.

Because the red cells of some of the donor dogs were hemolyzed directly *in vitro* without evidence of agglutination, it was desirable to determine if there was fixation of complement in this type of hemolysis. Considerable difficulty was experienced in the use of an isohemolytic system. This has also been the experience of others working with isohemolysins.[2] Inactivation of the complement in these sera by heating at 55°C. for 30 minutes caused a reduction of hemolysis of from one-half to two-thirds of that seen in the active controls.

*Conclusions.* Isohemolytic and isoagglutinative phenomena noted in occasional dogs during plasmapheresis, appear to be related to antigenic formation of immune bodies by the recipient due to certain factors in the donors' red cells. These hemolytic and agglutinative properties persist in the serum for long periods of time and may be intensified by renewed injection of red cells. Isohemolysis of the red cells of dogs appears to depend at least in part upon fixation of complement.

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## A Purified Diet Satisfactory for Growth, Reproduction and Lactation in Rats.

WARREN M. COX, JR. AND MIRIAM IMBODEN.

*From the Research Laboratory, Mead Johnson and Company, Evansville, Ind.*

The majority of investigative work on the adequacy or inadequacy of a given dietary regimen has involved principally 2 criteria: the rate of growth and the maximum adult weight attained. Osborne and Mendel[1] showed, on this basis, the improvement that had been accomplished in stock diets when growth records were compared with Donaldson's standards for the albino rat.[2] More recently, Bills' modification of the Steenbock diet* has been widely employed, and we have recently compared reproductive behavior

1 Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1926, **69**, 661.

2 Donaldson, H. H., *Wistar Institute of Anatomy and Biology*, Philadelphia, 1924, p. 275.

* Composition: Yellow maize, 57; whole milk powder, 25; linseed oil meal, 12; crude casein, 3.7; alfalfa leaf meal, 1.5; "iodized" table salt, 0.4; calcium carbonate, 0.4 %.