of estrogenic substance recovered or the severity of the symptoms. Positive spreads occurred with as little as 15 M.U.L., negative with as much as 200 M.U.L.

Of the 5 physiological menopause patients (ages between 44 and 59 years, duration 1 to 9 years) 2 showed a positive estrogenic blood reaction with 40 cc. of blood; 3 excreted from 60 to 400 M.U. per month. In only 2 (1 of which gave a positive blood reaction) was the excretion nil. The subjective symptoms of all 5 patients were severe.

The 3 X-ray castrates corresponded to the surgical castrates in their hormone titres.

Summary. The estrogenic factor continues to be excreted after surgical removal of the ovaries, as well as after the physiological menopause and X-ray castration. In spite of the presence of the estrogenic factor, excessive production of the gonadotropic factor (luteinizing and follicle stimulating) takes place.

No explanation of the source of the estrogenic factor, after the removal of the ovaries, can as yet be offered. Experiments to solve this problem are under way.

8472 C

Development of Incompatibilities in Dogs by Repeated Infusions of Red Blood Cells.*

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In the course of an investigation on the influence of diet upon the regeneration of serum protein it was found, as has been reported by others,[1] that with repeated plasmaphereses the hematocrit values tended to fall to anemia levels unless red blood cells from donors were injected periodically. Inasmuch as a fraction of the reinjected cells breaks down and liberates hemoglobin, which serves in the

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1 Holman, R. L., Mahoney, E. B., and Whipple, G. H., *J. Exp. Med.*, 1934, **59**, 251.

same capacity as dietary protein, an attempt was made to determine the degree of hemolysis *in vivo* in order to evaluate the liberated globin in terms of protein intake. Wu's[2] ultra-micromethod for the determination of hemoglobin was adapted, as modified by Bing[3] and McFarlane.[4]

In all of the 4 experiments performed, the determination of hemoglobin on the citrated plasma, saline washing, and the modified Locke's solution[5] in which the red cells were suspended for reinjection, indicated a remarkable uniformity in the hemolytic effects of these preliminary manipulations. The initial infusions of cells from the same donor (A) into 2 dogs (1 and 2) produced a proportional but negligible hemolysis. However, when cells from this donor (A) were injected a *second* time approximately 2 weeks later, a marked hemolysis *in vivo* was observed which failed to be related proportionally to the volume of cells injected.

TABLE I.

	Dog 1		Dog 2	
Experiment No.	I	II	III	IV
Cell volume injected, cc.	20	88	66	60
Maximum hemolysis *in vivo*, mg. %	5.8	187	15.5	52

Thus, in dog 1, the hemolysis, over and above a hemoglobinuria, was more than 30 times that recorded after the first cell infusion, although the cell volume injected was only 4½ times greater. In the case of dog 2, where similar cell volumes were injected, the maximum hemolysis was found to be more than 3 times greater subsequent to the second transfusion.

For the evaluation of proteins with respect to their ability to stimulate serum protein regeneration, 2 larger dogs (No. 3 and No. 4) were used. During the course of repeated plasmaphereses upon these animals we noted that the initial injections of cells from donors A and B were tolerated very satisfactorily. This was interpreted to be proof of compatibility, although recipients and donors were not typed. Preliminary cross-agglutination tests *in vitro* had indicated perfect compatibility of donor A with dogs 1 and 2. According to the literature, different blood types in dogs have been infrequently observed and these have been interpreted as of no clinical impor-

[2] Wu, H., *J. Biochem.* (Japan), 1922, **2**, 189.

[3] Bing, F. C., and Baker, R. W., *J. Biol. Chem.*, 1931, **92**, 589.

[4] McFarlane, W. D., and McKenzie Hamilton, R. C., *Biochem. J.*, 1932, **26**, 1050.

[5] Smith, H. P., Belt, A. E., and Whipple, G. H., *Am. J. Physiol.*, 1929, **52**, 54.

tance.[6] However, with repeated injections of the saline-washed red blood cells we have noted evidence of an incompatibility beginning with a mild hemoglobinuria, increasing in severity and resulting ultimately in profound shock. These infusions averaging 140 cc. of red blood cells in a total volume of 360 cc. were spaced from 7 to 10 days apart. In the interim the dogs were subjected to active plasmaphereses using homologous cells.

Ottenberg and Thalhimer[7] have produced an analogous picture in their study on cats. By repeated transfusions immune isoagglutinins and isohemolysins were developed in the recipients. Subsequent transfusions resulted in no unfavorable reactions other than a hemoglobinuria.

The development of the incompatibility in our experimental animals suggests a common mechanism—a sensitization induced in the recipient by repeated injections of an antigen in the red blood cells of the donor.‡ The clinical picture of the reaction obtained in dogs 3 and 4 is characteristic of anaphylactic shock.[8] There was intense salivation, vomiting, labored respiration, and involuntary urination and defecation while the injection was being made. The blood pressure was so very low that the femoral artery could hardly be palpated. The blood coagulability was so markedly reduced that hemorrhages from the sites of puncture in the femoral artery and jugular vein could be controlled only with great difficulty: tremendous hematomas were obtained in the thighs. Muscular weakness associated with a marked ataxia was common. Intensive hemoglobinuria was noted which continued until the animal's hematocrit approximated that recorded previous to the infusion. Indeed, dog 4 became markedly jaundiced, as evidenced by the intense yellow pigmentation of the cornea and skin.

The time between the collection and reinjection of the washed cells bore no relationship to the shock reaction; *i. e.*, homologous cells, even after being in the refrigerator 96 hours, were utilized satisfactorily, whereas heterologous cells, after a 3-hour period in the refrigerator, caused an immediate reaction.

The incompatibility was "one-sided" in that the sera of the recip-

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

[7] Ottenberg, R., and Thalhimer, W., *J. Med. Res.*, 1915, **3**, 213.

‡ We are greatly indebted to Dr. G. H. Smith, of the Department of Immunology, for suggesting a working hypothesis, and to Dr. W. M. Hale for his many helpful suggestions.

[8] Topley, W. W. C., *An Outline of Immunity*, Baltimore, Md., William Wood and Co., 1933, pp. 194-195.

ients agglutinated the cells of the donors, whereas cells of the recipients were compatible with the sera of the donors.

The question arose whether the recipients were sensitized specifically against the present donors, against all dogs or against certain dogs, the cells of which have an antigenic make-up similar to that of the original donors. To investigate this, we carried out microagglutination tests *in vitro,* employing the cells of 25 dogs chosen at random and the sera of dogs 1, 3 and 4. The following conclusions seem warranted:

1. The sensitized dogs were incompatible with 50% of the dogs tested, although none of the entire group tested had ever served as donors. The suggested explanation is that these non-compatible animals have an antigenic make-up in their red blood cells similar to that of the original donors. Successful transfusions into the sensitized dogs were subsequently made with the cells from those animals that were shown to be compatible by *in vitro* tests.

2. The compatibility of the cells of any given dog with the sera of our recipients is independent of breed or sex.

3. Dogs 3 and 4, having been sensitized against the cells of both donors A and B, reacted in exactly the same manner to the cells of all animals tested. In the case of dog 1, which had been sensitized only against the cells of dog A, we also obtained a similar response. Apparently both dogs A and B have in their red cells a common antigen, which is possessed also by approximately 50% of the dogs.

4. Further evidence for initial compatibility was observed when dogs representative of each of the 2 groups were cross-typed.

A passive transfer test was made employing a dog characteristic of that group still compatible with the sensitized dogs. This animal, having never been the recipient of cells from the original donors, was shown by tests to be compatible with them. Plasma to the extent of approximately one-fourth of the animal's blood volume was injected, citrate being used as the anticoagulant. On the following day the animal evidenced *in vitro* an incompatibility with the donors. Intravenous injection of a cell suspension from donor A produced a syndrome suggesting mild anaphylaxis. Although we failed to observe the clinical symptoms of shock, the animal showed a marked nystagmus which persisted 2-3 minutes. The normal coagulation time of this dog's blood was 2 minutes; after the injection of the cells the coagulability was so markedly decreased that the blood failed to clot even after 6 hours. A test made 18 hours later showed normal coagulation time. The per cent

eosinophiles did not vary but remained at the normal level of 2-3%.§ With the next urination a marked hemoglobinuria was noted which persisted for more than 48 hours.

Another dog, belonging to that group which was incompatible with the sensitized dogs, received an injection of plasma (citrated) which was approximately one-fifth of its blood volume. This injection was alone sufficient to cause an intense hemolysis *in vivo* with a corresponding hemoglobinuria. This harmonizes with the observation of hemolysis in our recipients (dogs 3 and 4). The hemolysin which developed in these animals acted to destroy all of the donor's cells. By reversing the situation, *i. e.,* by injecting the sensitized serum into the blood stream of an incompatible dog, all of the cells of which were "foreign" to the hemolysin, an immediate and continuous hemolysis took place. Thus, whereas the normal hematocrits of this dog averaged 48%, the hematocrit on the day subsequent to the injection was 22%. The cell volume for the next 2 days approximated the same low value and was associated with an intense hemoglobinuria. The animal was beginning to show the characteristic yellow pigmentation of jaundice. The dog was found dead on the following day. Necropsy did not reveal any abnormalities other than paleness of the visceral organs characteristic of anemia.

In the course of our investigations we have noted repeatedly that the compatibilities between the recipients and donors were restored within 5-10 weeks after the last infusion of cells. These were evidenced both by agglutination tests *in vitro* and the performance of successful infusions of cells obtained from these donors. However, within the following week agglutinations *in vitro* were observed when cell suspensions of the donors were added to the sera of the recipients. These phenomena are truly characteristic of anaphylaxis.[9]

Five dogs of the compatible group have been subsequently employed as blood donors, enabling us to continue our original investigation. The sensitized dogs have received from these donors a total of 28 infusions of red blood cells spaced for the most part 7-14 days apart. No evidence of incompatibility was observed subsequent to these injections, nor have we noted the appearance of any agglutinins for the cells from these donors. The non-development of

§ Thanks are due to Dr. J. M. Orten for the determinations of the coagulation time of the blood and for the differential white cell counts.

[9] Zinsser, H., and Bayne-Jones, S., *A Textbook of Bacteriology with a Section on Pathogenic Protozoa,* New York, N. Y., D. Appleton-Century Co., 1935, p. 258.

antibodies for these cells after the numerous infusions is indicative that the present donors will remain compatible with the sensitized dogs.

Inasmuch as dogs, for all practical purposes, are of the same group, so that any dog may be a suitable donor for another, the development of the incompatibilities in this species bears a striking similarity to the clinical picture obtained in some cases with humans. Wiener[6] states that "in cases where the same patient is to be given more than one transfusion, the cross match should be repeated before each transfusion, even if the same donor is used again. Several cases have been reported in which, although the first transfusion was successful, reactions took place during subsequent transfusions where the same donor was used. . . . . This subject has not been investigated thoroughly and deserves further study."

8473 C

Comparative Minimal Hypnotic Effects, Toxicity, and Pathology, Produced by Sodium and Magnesium Salts of Phenobarbital.

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Gilman and Barbour[1] have shown that the minimal hypnotic dose (M.H.D.) and the minimal lethal dose (M.L.D.) of magnesium phenobarbital when administered orally to rats are approximately 25 mg./kilo and 275 mg./kilo, respectively. According to the criterion of Issekutz[2] this would suggest a "hypnotic range" of 250 mg./kilo in the rat, or 10 times the M.H.D.

In a series of 232 rats we have found that when sodium phenobarbital and magnesium phenobarbital in freshly made 4% solutions are administered subcutaneously to rats in parallel experiments that there is no essential difference in the toxicity of either salt. The M.L.D. of both salts for rats by the subcutaneous route is close to 215 mg./kilo.

In dogs, the M.L.D. of both the sodium and the magnesium salts

1 Gilman, A., and Barbour, H. G., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1634.

2 Issekutz, B., *Therap. Monatshefte*, 1915, **29**, 379.