As stated above, the purified enzymic preparation renders heat-killed pneumococci Gram negative, whether the bacteria are R or S variants, and irrespective of type-derivation. The cells, however, do not undergo dissolution but retain their characteristic morphology; the turbidity of the bacterial suspension is also very little altered.

Following the repeated intravenous injection of heat-killed pneumococci Type I which have been digested with the enzyme, no precipitins for the capsular polysaccharide appear in the serum, while these invariably occur if the untreated cells are injected. It appears, therefore, that the enzyme has the property of inactivating the capsular antigen of virulent pneumococci.

The enzyme, however, does not decompose the capsular polysaccharide itself. In fact of all the soluble substrates tested, yeast nucleic acid* was the only one to be attacked by the purified preparations. A description of this reaction is presented elsewhere.[2]

It is interesting to point out that some preparations of crystalline trypsin† and chymotrypsin† were found to exhibit a small measure of activity against the Gram positive structure of pneumococci and against yeast nucleic acid. After repeated recrystallizations, however, the proteolytic enzymes no longer have any action upon either of these substrates.

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Differentiation of Blood Groups in Dogs Based on Antigenic Complexes Present in the Erythrocytes.*

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When dogs are subjected to prolonged, intensive plasmapheresis, the hematocrit values tend to fall to anemia levels in spite of the

* The authors are indebted to Dr. P. A. Levene for a sample of yeast nucleic acid.

2 Dubos, R., *Science*, 1937. In press.

† The authors are indebted to Doctors J. H. Northrop and Dr. M. Kunitz for supplying several samples of crystalline trypsin and chymotrypsin.

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† Alexander Brown Coxe Fellow, 1936-37.

reinjection of the cells removed in the bleedings of the previous days. To compensate for this destruction of erythrocytes *in vivo,* it is necessary to inject periodically red blood cells obtained from donor animals. Although dogs are generally compatible with each other as regards blood transfusions[1] and this is evidenced by successful initial injections of cells, subsequent transfusions may create a sensitization which renders the donors incompatible thereafter.[2-5] The development of isoagglutinins and isohemolysins results in the production of an intense hemoglobinuria; the continuation of the cell infusions, spaced from 7 to 10 days apart, is followed by symptoms of profound shock.

It was suggested[3] that the development of this incompatibility is due to a common mechanism—a sensitization induced in the recipient by repeated injections of an antigen in the red blood cells of the donor. The evidence for this belief may be summarized as follows:

(1) The clinical picture of the reaction obtained in our dogs is characteristic of anaphylactic shock in that species.

(2) With the lapse of time (5 to 10 weeks) there is a restoration of compatibility. However, in this condition the sensitiveness may easily be made manifest again within one week by injection of the antigen.

(3) The incompatibility is "one-sided" in that the sera of the recipients agglutinate and hemolyze the cells of the donors, whereas cells of the recipients are compatible with the sera of the donors.

(4) The incompatibility can be passively transferred to a normal non-sensitized dog.

(5) Finally, the sensitized animals when in shock respond immediately and favorably to the intracardiac administration of adrenalin.

When the sera of the sensitized dogs were subjected to microagglutination tests *in vitro,* employing the cells of 36 dogs chosen at random, we noted that approximately 50% of these animals were incompatible with the sensitized dogs although none of the entire group had ever served as donors. This incompatibility, which proved to be independent of breed or sex in our series of animals, is probably due to these particular dogs having an antigenic make-up in their red blood cells similar to that of the original donors. To

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

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

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

4 Wright, A., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 440.

5 Melnick, D., and Cowgill, G. R., *Am. J. Physiol.,* 1937, **119**, 70.

facilitate discussion the symbol, α, has been assigned to that group still compatible with the sensitized dogs; the symbol, β, to the incompatible group.

Five dogs of the compatible group, α, were subsequently employed as blood donors, enabling us to continue our studies of serum protein regeneration. Two of the sensitized dogs (α dogs) have received to date from these donors a total of 38 infusions of red blood cells, spaced for the most part 7-14 days apart. No evidence of an incompatibility subsequent to these injections has been observed, nor has the appearance of any agglutinins for the cells from these donors been noted. The non-development of antibodies for these cells after the numerous infusions indicates that the present donors will remain compatible with the sensitized dogs. Apparently these donor animals have an antigenic grouping in their red blood cells similar to that of the recipients.

We are now in a position to report further observations on blood grouping in dogs by carrying out experiments which are essentially the reverse of those described above. These were made in the course of our present investigations of serum protein regeneration.

Dog No. 3, described in a previous communication[3] and a member of the α group, received 150 cc. of cells obtained from an animal previously demonstrated to be a member of the incompatible (β) group. Within the following week marked agglutination *in vitro* was noted when cell suspensions of this donor were added to the sera of the recipient. This sensitized animal has been used as an indicator to enable us to group satisfactorily all dogs used in our subsequent studies.

Three dogs of the β group, this time acting as recipients, have received 16 injections of cells from 4 donors of the β group without developing any signs of an incompatibility. One of these animals actually received a total of 11 cell infusions. Micro-agglutination tests also indicated the complete absence from the sera of these recipients of agglutinins for cells of the β group. Apparently all of these dogs, members of the β group, possess the same antigenic complex in their red blood cells. These results are analogous to those obtained when both recipient and donor were of the α group.

Four dogs of the β group were subjected to 35 cell injections from 5 animals of the α group with no untoward effects being manifest. The sera of the recipients were also observed to contain no agglutinins or hemolysins for the cells of the donors. These findings are in direct contrast to those noted when conditions were reversed, namely, when the recipients were members of the α group and the donors of the β group. Apparently the erythrocytes

of dogs of the β group possess all of the antigenic groupings present in the cells of animals of the α group and in addition contain some other complex which may act as a foreign body when injected into dogs only of the α group. Therefore, if it is true that 50% of the dogs belong to either the α or β group and if these are the only 2 groups present in that species, it follows that there is only one chance out of 4 that an incompatibility will occur when a dog is subjected to repeated infusions of red blood cells from a donor animal. This is obvious when reference is made to the table which summarizes our findings. It may be for this reason that in Whipple's laboratory,[4] where animals were subjected to repeated injections of erythrocytes from a group of donor animals, hemoglobinuria and shock were observed with some of the dogs whereas others received similar exchanges of red cells from the same group of donors without untoward effects.

TABLE I.
The Results of Repeated Injections of Red Blood Cells into Dogs.

Blood Group of		
Donors	Recipients	Results
β	α	Blood incompatibilities develop in recipients
α	α	Dogs remain compatible.
β	β	" " "
α	β	" " "

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Respiratory Effects of Substituted Phenols at Varying Carbon Dioxide Tensions.

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During a study of the reversible inhibition of cell division which is produced in fertilized marine eggs by certain nitro and halophenols[1, 2] the CO_2 tension was found to be a significant factor in the degree of inhibition attainable.[3] The respiratory effects of substituted phenols were then measured on cells which were exposed to a gas phase containing oxygen and CO_2 in various ratios.

1 Clowes, G. H. A., and Krahl, M. E., *Science*, 1934, **80**, 384.

2 Clowes, G. H. A., and Krahl, M. E., *J. Gen. Physiol.*, 1936, **20**, 145.

3 Krahl, M. E., Clowes, G. H. A., and Taylor, J. F., *Biol. Bull.*, 1936, **71**, 400.