ported rat blood volumes obtained by the dye method, it is not possible to draw any conclusions as to the factors responsible for the difference between these data and that yielded by transfused tagged cells.

Summary. 1. The mean blood and packed cell volumes of 22 adult male rats, as measured by transfused tagged cells, was found to be 4.95 and 2.32 ml per 100 g of body weight respectively.

2. The mean blood and packed cell volumes of six of the 22 rats, assayed by the hemoglobin extraction method, were found to be 4.92 and 2.32 ml per 100 g of body weight.

3. The efficiency of perfusion for the total rat was 95%. This corresponds to a possible error in blood volume, introduced by removal of tagged transfused cells from the recipients circulation, no greater than 5%.

4. Comparison of the blood volume of various tissues taken from maximally bled rats with the blood volumes of similar tissues taken from perfused rats indicates that perfusion is least effective for spleen and most effective for kidney and brain.

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Isoimmunization in the Pig Following Multiple Transfusions of Incompatible Blood.* (18015)

WILLIAM J. KUHNS.† (Introduced by M. M. Wintrobe)
From the University of Utah College of Medicine, Department of Medicine, Salt Lake City, Utah

The existence of 4 distinct blood group systems in the pig has been described(1). These have been conveniently designated as Pi groups 1, 2, 3 and 4 (Pi_1, Pi_2, Pi_3, Pi_4). The Pi_1 group gave the consistently strongest reactions and was dominant in this respect over the other 3 groups. Members of Pi_1 possessed either the homologous antigen or antibody, or, in 15% of animals studied, carried neither antigen nor antibody (O_1). Members of Pi_2 were similar in this respect except that (O_2) was more common than the homologous antigen or antibody (77.9%). There was a reciprocity between the Pi_1 and Pi_2 antigen-antibody components when they occurred together. Occasionally an animal was O for both groups. Further isoagglutination and absorption experiments demonstrated the existence of two additional reciprocally related systems (Pi_3 and Pi_4) which were somewhat analogous to the A and B blood groups in man. On the basis of these groups, it was possible to predict the existence of fourteen blood group combinations.

In the present report, 2 pigs of anti-Pi_1 specificity were injected with serial blood transfusions from one pig of Pi_1 specificity over a period of 43 days in order to determine the immunizability of the recipient pigs against this blood group antigen. This was done with a view to utilization of the pig as an experimental animal in the production of experimental hemolytic syndromes. Young(2) has reported similar procedures in the dog.

Methods. The schedule of isoimmunization was so arranged that citrated whole blood from the donor pig (Pi_1) was given intravenously by syringe to 2 recipient pigs (anti-Pi_1) in amounts which increased progressively from 7.5 ml to 15.0 ml every 2 to 3 days over a period of 26 days. Following this time, the quantity of blood given at any one time was

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† Post-Doctorate Fellow of the United States Public Health Service. Present address: New York University College of Medicine, Dept. of Microbiology.

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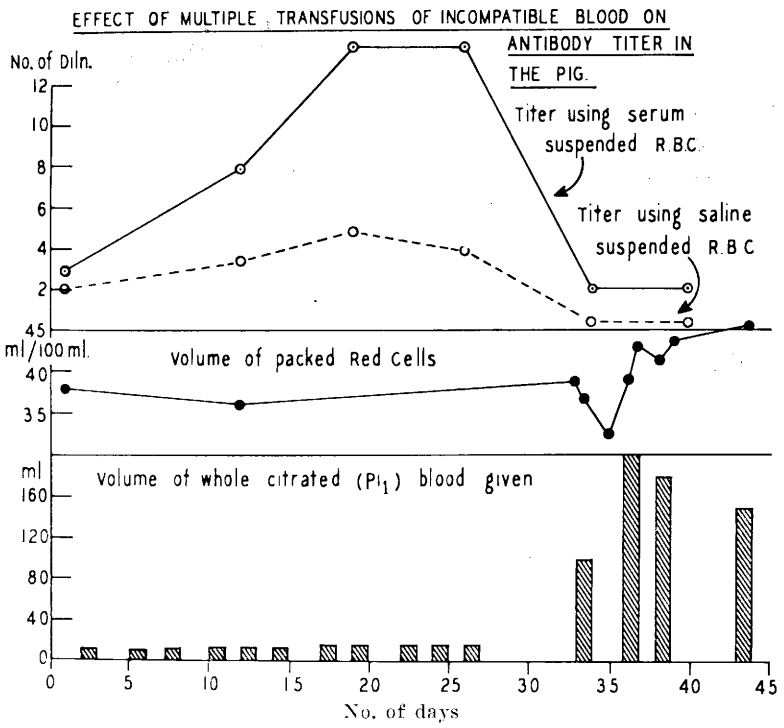

FIG. 1.

increased, so that amounts which varied from 100 to 200 ml were given in a total of 4 infusions over an 18 day period. Frequent whole blood samples were withdrawn for the determination of the volume of packed red cells, icterus indices and antibody titrations. All titrations were performed utilizing both saline and "blocking" technics. Determinations of "blocking" antibodies were made by serially diluting the recipient pigs sera (anti-Pi_1) with pooled Pi_1 sera, and adding equal amounts of serum suspended Pi_1 red cells to each dilution. Saline titrations were performed in similar fashion with the exception of the change of diluent.

Results. The results in one recipient pig are indicated in Fig. 1. Analogous results were obtained in the second transfused pig. There occurred no consistent fall in the volume of packed red cells during the total period of isoimmunization, nor did the icterus indices rise to abnormal levels at any time. Although titrations of the sera for anti-Pi_1 antibodies showed no demonstrable increase in titer in saline suspended Pi_1 red blood cells during the total period of study, the titer of "blocking" antibodies of anti-Pi_1 specificity in both animals increased to a marked extent during the same time. However, following the infusion of large quantities of blood the titers practically disappeared, along with a transient fall, followed by a progressive increase in the volume of packed red cells.

Discussion. It was of interest that isoimmunization of 2 pigs against a naturally occurring blood group antigen (Pi_1) resulted in the formation of "blocking" or incomplete antibodies of high titer against Pi_1. The sera of both animals prior to transfusion yielded approximately the same low titers in both serum and saline suspended cells, which were interpreted as indicating the presence of naturally occurring anti-Pi_1 agglutinins. However in the course of the Pi_1 transfusions there appeared the so-called "immune" anti-Pi_1 antibodies of the "blocking" variety in high titer (demonstrable in serum suspended Pi_1 red cells) while the saline titers showed no appreciable change (Fig. 1). This situation is some-

what analogous to the findings of Witebsky (3), Ervin and Young(4) and Wiener(5) in man where incompatible transfusions or heterospecific pregnancies involving the A-B-O blood groups occasionally result in the formation of "blocking" or incomplete antibodies against blood groups A or B. The "blocking" antibody in turn is of importance in the pathogenesis of erythroblastosis fetalis and in transfusion reactions.

Whether selective fetal death in the pig is ever the result of transplacental isoimmunization and the formation of "blocking" antibodies, is unanswerable at the present time. The studies of Corner(6), however, may be pertinent in this regard, and his concept of embryonic morbidity in mammals as a sequel to internal defects of the germ cells may profitably be re-investigated in the present light of demonstrable blood group incompatibilities in many mammals, including the pig.

Hemolysis in the transfused pigs was apparently not great as judged from the serial icterus indices and volume of packed red cell determinations. However, the initial decrease in volume of packed cells which took place shortly after the titer decrease suggests a possible transient hemolytic episode because of antibody absorption by Pi_1 red cells. This point will require further investigation in view of the fact that Ashby studies were not performed, and urobilinogen excretion in urine and stools was not determined.

That hemodilution was the most likely basis for the sudden titer decrease is suggested by the fact that blood typing of the anti-Pi_1 pigs subsequent to the transfusion of large volumes of Pi_1 blood revealed considerably weaker agglutination than was present prior to the experiment. In addition, there occurred a progressive concomitant rise in the volume of packed red cells after the initial temporary fall.

Summary. Multiple transfusions of Pi_1 blood from one pig into two other pigs of anti-Pi_1 specificity resulted in the formation of high titers of "blocking" antibodies of anti-Pi_1 specificity. The implications in experimental hemolytic syndromes and in selective fetal death in the pig are discussed.

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An Improved Assay for "Strepogenin" Based on Essential Nature of Material for *Lactobacillus bulgaricus* 09. (18016)

LEMUEL D. WRIGHT, JOSEPH S. FRUTON, KATHERINE A. VALENTIK AND HELEN R. SKEGGS

From the Medical Research Division, Sharp and Dohme, Inc., Glenolden, Pa., and the Department of Physiological Chemistry, Yale University, New Haven, Conn.

"Strepogenin" has been applied as a name to a factor or group of closely related factors essential for the growth of certain Group A streptococci(1,2) or stimulatory for the growth of a variety of lactic acid bacteria (2-4). Partial hydrolysates of certain purified proteins have been shown to have high "strepogenin" activity(2,4,5). Recent work has suggested that the amino acids serine,

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