Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 4, Number 1, January, 1935.

Blood Groups of the Horse with Special Reference to their Significance in Blood Transfusion and in Horsesickness Immunisation.*

By P. J. J. FOURIE, M.R.C.V.S., Dr. Med. Vet. (Utrecht), Sub-Director of Veterinary Services, Onderstepoort.

INTRODUCTION.

Blood transfusion is a therapeutic measure which has a wide application in human medicine. In veterinary medicine it has, in general, a much more limited application. In the production of certain biological products, particularly antisera, transfusion of large amounts of blood is frequently necessary. This is the case when hyperimmunisation is practised against horsesickness during the routine preparation of horsesickness antiserum, for the ordinary immunisation of horses and mules against this disease in South Africa. During hyperimmunisation as much as 10 litres of blood are transfused at one time and during ordinary immunisation up to 400 c.c. of antiserum are injected intravenously. Both during or soon after hyperimmunisation or ordinary immunisation a variable number of animals develop certain symptoms which are generally interpreted as those of shock. These symptoms are undoubtedly anaphylactoid in character and a study of blood groups in horses was undertaken to determine what part, if any, blood group incompatibility played in their production.

LITERATURE.

For a discussion of the whole blood group question with a complete list of references the " Handbuch der Blutgruppenkunde " (1932) should be consulted. Herlyn (1928) summarised the available information up to that date referring particularly to blood groups of the domesticated animals, and Doan (1927) gave a complete review of the transfusion problem.

Landois is quoted by Schermer and Hofferber (1928) to have described individual reactions of normal blood in 1875 without, however, recognising the specificity of these reactions.

Hesch (1932) points out that previous to Landsteiner's classical description in 1901 of three blood groups in the human subject, Shattock described in 1899 and in 1900 agglutination of the red cells of certain individuals by the sera of others. As these sera originated from persons suffering from various diseases, the phenomenon was interpreted as being of pathological significance. Landsteiner himself also observed this in 1900, when working with sera of diseased

* This paper was completed on 22nd February, 1934.

persons. In addition to the three groups (O, A and B) described by Landsteiner in 1901, Decastello and Sturli, co-workers of Landsteiner, described the fourth group (AB) as the exception to Landsteiner's three-group classification. This was for the first time recognised as the fourth physiological group in 1906 by Jansky. Moss in 1910, working independently and without knowledge of Jansky's work, also described this as the fourth group. Ehrlich and Morgenroth (1900) differentiated between isolysins and autolysins of goats.

Although Klein showed as early as 1902 that the serum of one horse may agglutinate the red cells of another, very little progress in the study of blood groups in the domesticated animals was made for a number of years. As far as can be ascertained from the available literature, definite groups in the domesticated animals were first described by Ottenberg and Friedman in 1911. They found three groups in cattle and four groups in rabbits. However, in this connection it should be stated that Von Dungern and Hirschfeld (1909) were able to demonstrate the presence of iso-agglutinins and group characteristics, by means of injecting the blood of a dog into other dogs, and in a later publication (1910) they, in addition, made use of absorption tests and were able to show, inter alia, that the red cells of most animals will absorb the β agglutinin of human sera.

Brockman (1911) carried out extensive absorption tests with human blood and blood from dogs, bovines, rabbits and birds, but did not describe groups in any of these animals.

Ingebrigsten (1912) examined the blood of 40 cats. He found iso-agglutination, but this occurred in such an irregular manner that grouping was not possible. It is of interest to record that even at that early date the main object of Ingebrigsten's study of blood groups in the cat, was to find out if the successful transplantation of arteries was in any way affected when using a donor which does not belong to the same blood group as the recipient. He found no evidence establishing any such relationship.

Fishbein (1913) could demonstrate the presence of iso-agglutination in sheep, swine, cattle and rabbits, but was not able to classify his results into well-defined groups. He also states that he found iso-agglutination in dogs, but no figures for the reactions in dogs were reproduced.

According to Thomsen (1932) a number of authors, e.g. Weszeczky (1920), Panisset & Verge (1922), Walsh (1924), found irregularities in iso-agglutination in the horse and, although their results suggested group differences, these were not actually classified. This, according to Thomsen, was first described by Hirszfeld and Przesmycki (1923), who found four main groups, as they occur in the human subject, but in addition found some individuals which could not be so grouped. (Hirszfeld and Przesmycki had already in 1921 examined 45 horses for iso-agglutination and were able to differentiate two factors A and B—A was found in 70 per cent., B in 15 per cent. and O in 9 per cent. of the animals examined).

This was confirmed by Landsteiner and Van der Scheer (1924), Schwarz (1926), Newodoff (1927) (4-6 per cent. of his horses could not be grouped), Schermer (1928) (16 per cent. of his horses could not be grouped), and by Anderson (1932). Schermer and Hofferber (1927) described five main groups and six sub-groups. It would appear that these authors were not convinced by the absorption tests they carried out that there are only two factors A and B in horse blood. Thomoff (1930) described four groups and maintained that with the precise and exact technique employed by him sub-groups can be excluded, and further that it is possible with two specially selected normal A and B sera to determine the group of any particular horse.

TECHNIQUE.

It seems to be generally agreed that, owing to rouleaux formation (pseudo agglutination) considerable difficulty is experienced in accurately determining undoubted agglutination when mixing serum and red cells of horses. On this account the glass slide method, originally and mainly employed in human blood work, was found to be unsuitable. When a large number of determinations have to be made in one day, the additional factor of evaporation in dry climates offered almost insuperable difficulties to the general application of this method. Schwarz (1926) mentions that considerable care is necessary in distinguishing rouleaux formation from true agglutination, when using the hanging drop method. For these reasons the centrifuge method, as described by Schiff (1929), was used with satisfactory results.

There is a conspicuous lack of uniformity in the details with which the various workers carry out the test tube method of determining iso-agglutination in horses, particularly in the proportions of red cells and serum or plasma employed.

Schwarz (1926) mentions that serum from defibrinated blood is unsatisfactory, as a certain amount of haemolysis is unavoidable, and thus prefers citrated plasma. However, serum obtained after allowing the blood to clot may, according to him, be used, but he finds that serum may, from time to time, give uncertain reactions when tested against some red cells, whilst with plasma definite reactions are always obtained. He uses ·95 c.c. plasma with ·05 c.c. of a 25 per cent. red cell suspension, allows this to stand for half an hour at 37° C. and reads the reactions without spinning, after allowing the mixture to stand for 12 hours at room temperature. Equally good results are obtained by washed and unwashed corpuscles.

Schermer and Hofferber (1927-1928) use ·1 c.c. of serum to ·2 c.c. of a 1-2·5 per cent. washed red cell suspension. These are the same quantities recommended for use in the human being (Schiff, 1929). In a later publication, Schermer (1928) recommends the use of a 1 per cent. suspension of washed red cells. After thoroughly mixing the serum and the red cells, the mixture is allowed to stand for five minutes and then spun for two minutes at 1,500-2,000 revolutions per minute. Two further tubes are prepared

in a similar way, one is allowed to stand for 12 hours at room temperature, and the other is incubated at 37° C. The use of the last-named tube is especially designed to exclude pseudo-agglutination. A fourth examination (microscopic) is made in hanging drops. They regard their method as superior to those of Rohdenberg; Panisset and Verge, Bialosuknia and Kaczukowski, and that of Groll, details of all of which are quoted by them.

Thomoff (1930) uses ·2 c.c. of serum and ·2 c.c. of a 1·5 per cent. suspension of washed red cells; one tube is incubated at 37° C. and read after 2 hours, and a second tube is kept at room temperature (22-24° C.) and read after 4 hours. In neither case is the centrifuge used. In addition to these, he uses the glass slide method macro- and microscopically and finally makes a haemolytic test.

Anderson (1932) uses ·1 c.c. of serum with ·2 c.c. of a 2 per cent. suspension of washed red cells. Results are read after incubating for 12 hours at 37° C.

Burghardt (1933) uses a concentrated serum which is obtained by a method of freezing. This serum should have three times the agglutinating value of ordinary serum and he maintains that compared with normal serum 11 per cent. more positive reactions are obtained. He mixes ·2 c.c. of a suspension of ·075 c.c. red cells in 10 c.c. physiological saline and ·1 c.c. concentrated serum. Results are read after one hour and confirmed after 15 hours without spinning.

All workers are agreed that the concentration of agglutinins in horse serum is low. Varying proportions of serum and red cell suspension were therefore tried out, the best results being obtained by using ·5 c.c. (10 drops) of serum to ·1 c.c. (2 drops) of a 1 per cent. suspension of pure concentrated red cells in Ringer-Locke solution. As a particular red cell suspension was often tested against as many as 47 sera at the one time, the pipettes used for the red cell suspension (47 × 2 = 94 drops being used) were much larger than those used for the serum (10 drops only being used), so that, actually, the red cell drops were larger than the serum drops and the true proportions probably were 10 drops of serum to 3 drops of equal size of a 1 per cent. suspension of red cells. After suitably shaking the mixture to ensure thorough distribution of the red cells, the tubes were allowed to stand for at least 5 minutes at room temperature and then spun at 2,000 revolutions per minute for 3 minutes.

Collection and Preparation of Serum.

The 47 horses examined for blood groups were bled from the jugular vein into wide-mouth flasks. On separation from the clot the serum was carefully poured off into sterile flasks, and after allowing the serum to stand in these flasks for 24 hours, it was transferred to a third set of flasks. During this transfer it was filtered through several folds of sterile butter muslin. With ordinary care no difficulty was experienced in obtaining a clear sterile serum completely free from red cells.

During the initial stages of this work an attempt was made to preserve the serum. The preservative advocated by Schiff (1929) nearly always produced a precipitate with horse serum. Glycerine or biniodide of mercury caused haemolysis; the perchloride of mercury destroyed the agglutinins. Boracic acid has been successfully employed as a preservative for whole blood, the serum from which being used for agglutination tests (Baker, 1925) and complement fixation work (Robinson-Pers comm.). A 5 percent. solution of boracic acid appeared to give excellent results and, in the first series of tests carried out, such preserved sera were actually used. However, when nearly half the number of horses were worked through, auto-agglutination was found in one horse (20232). As the possibility of a mistake in the numbering of the flasks could not, with certainty be eliminated, the 47 horses were again bled, and the tests repeated with unpreserved and with boracic acid preserved sera. It was then clearly shown that the boracic acid serum of horse 20232 agglutinated its own red cells, whilst the sterile serum tested at the same time against the same red cells did not do so. The boracic acid serum of horse 20232 agglutinated the red cells of 13 out of 16 horses, against which it was tested. The unpreserved serum of this horse apparently did not contain any agglutinins, as no agglutination was found when it was tested against the red cells of some 50 horses. In view of these results boracic acid was discarded as a preservative.

Superol (chinosol) recommended by Van Herwerden and Boele-Nyland (1930) for the preservation of red cells, did not in any way interfere with agglutination. It may cause a greenish discolouration of horse serum, possibly due to the presence of bile salts present in the normal serum of this animal.

As there was no great difficulty in maintaining a supply of sterile serum, serum preserved with chinosol 1:1000 was only occasionally used. The 47 animals were all bled on one day and within a week clear sterile serum from each animal, suitably bottled, was stored in the ice chest for use as required.

Collection and Preparation of Red Cells.

Only small amounts of blood were necessary. The red cells of 4-6 horses were tested every second or third day against the sera of all the 47 horses used. A convenient amount, usually 20-30 c.c. of whole blood was collected direct from the jugular vein into sterile centrifuge tubes. These were immediately centrifuged at 3,000-4,000 revolutions per minute for 5 minutes. By this time the plasma might or might not have coagulated, but this was of no consequence, as, by that time, the red cells in all cases had sedimented. The mouth end of a sterile 1 c.c. pipette was closed with the finger (to prevent the entrance of any plasma into the free end of the pipette) and the pipette passed along the side of the centrifuge tube into the mass of sedimented red cells; 0·5 c.c. was removed and suspended in 50 c.c. of warm (±35° C.) sterile Ringer-Locke solution. Using these proportions approximately a 1 per cent. suspension of red cells was obtained. The red cell suspension was usually made at about 9 a.m. and was used on the same day between 2-4 p.m. A red cell suspension older than 12 hours was never employed.

Schermer and Hofferber (1928) maintain that better reactions are obtained by using red cells which have been kept for 24-28 hours than by the use of freshly-prepared suspensions. Provided a control test is carried out to exclude auto-agglutination caused by bacteria (Thomsen hemagglutination phenomenon) no serious objection can be raised to this.

GROUP CLASSIFICATION OF A MIXED LOT OF SOUTH AFRICAN HORSES.

The results obtained by testing the red cells of 47 horses against the sera of the same animals for iso-agglutination are recorded in Table 1.

The designation + is used for well-marked iso-agglutination. If the tube is shaken after spinning, the clumped red cells will remain unbroken and can be made to swirl through the serum en masse; however, if it does become broken up by vigorous shaking, a number of individual granules will still be apparent, the serum remaining clear.

The designation + + is used for those reactions where with fairly vigorous shaking the clumped red cells are broken up into numerous very fine particles, but the serum remains clear and a true suspension of the red cells does not occur. This can be regarded as a weak iso-agglutination.

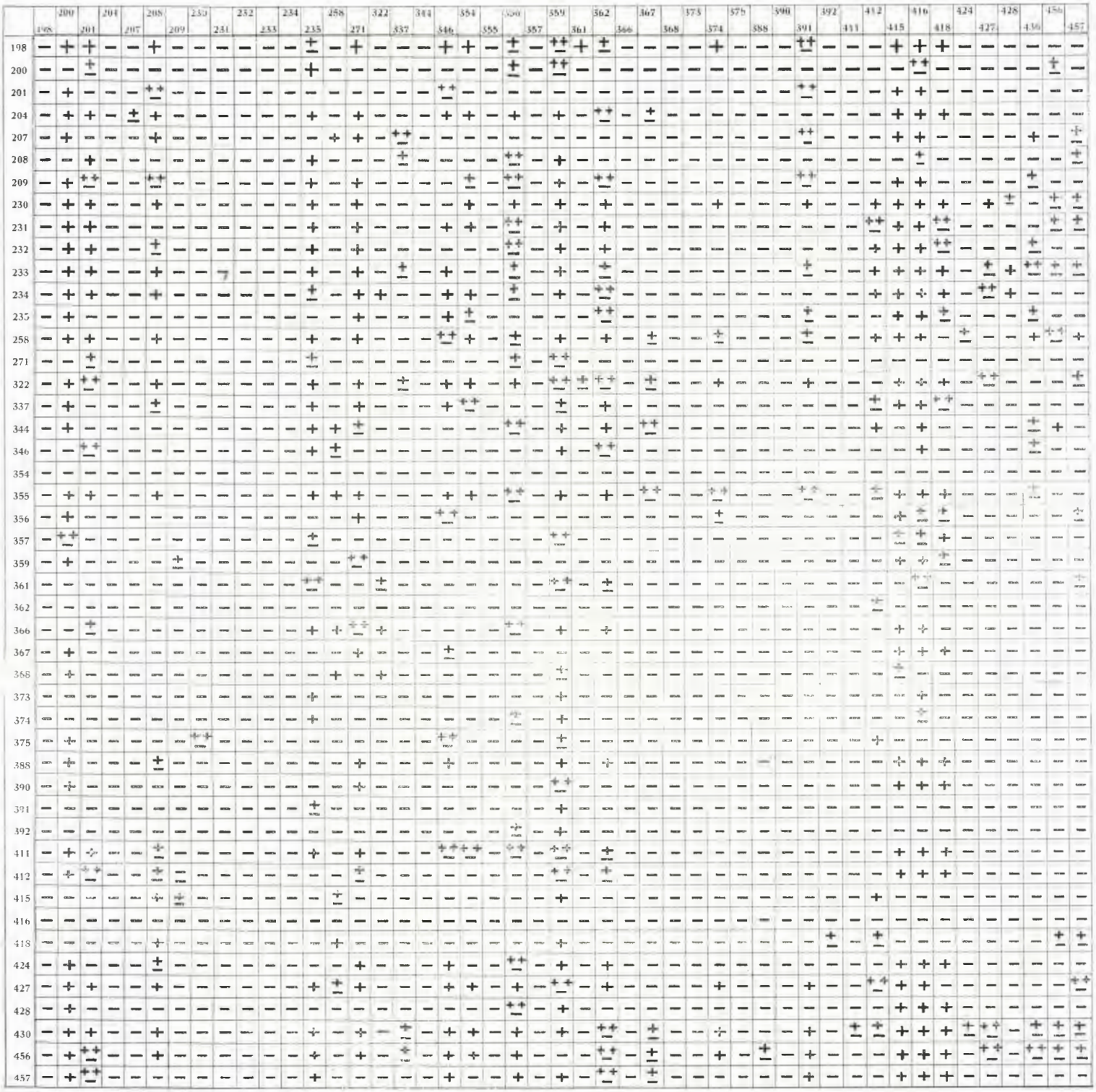
The designation ± indicates a slight clumping noticeable on first shaking the tube, but a uniform suspension is effected without any difficulty on further shaking. This type of reaction is possibly associated with pseudo-agglutination.

The designation – denotes the absence of any agglutination; when the tube is agitated a uniform suspension immediately results.

On referring to Table 1 it will be seen that most animals, of which Nos. 20198, 20204, 20207, 20209, 20231, 20232, 20233, etc., are examples, have group characteristics like that of AB in the human subject. The sera of these animals do not produce iso-agglutination of the red cells of any of the horses against which they were tested. Their red cells on the other hand are agglutinated by the sera of nearly all the other horses used. Their red cells will, of course, not be agglutinated by sera of animals belonging to the same group, and this largely explains the frequency with which the absence of iso-agglutination was recorded. Approximately 50 per cent. of the 47 horses used belong to this group AB, if the agglutinin factor only (serum factor) is considered. To conform to the group requirements of AB in the human subject, the red cells of these animals should be agglutinated by the sera of all the other groups. However, in this respect there are noticeable irregularities, e.g. the serum of horse 20258 agglutinates the red cells of horses 20207, 20344, 20355, 20366, and 20368. All these animals would, in so far as the agglutinin factor is concerned, belong to group AB. But serum 20258 reacts negatively to the red cells of horses 20198, 20204, 20209, 20230, 20231, 20232, etc., animals which would also

TABLE 1.

SERA.



In this table the first two figures of the numbers of all the horses were omitted as these figures are common to all the horses. The numbers should therefore read: 20198, 20200, 20201, etc.

13–14

appear to belong to the same group AB. This irregularity requires further study, a possible explanation being that the B agglutinogen of these horses may be present in such small quantity that with the technique employed, it does not react with the serum of horse 20258, if it is assumed, for the moment, that this horse belongs to group A. In this instance a deficiency of the red cell factor (agglutinogen) is postulated. This has, on several occasions, been reported in the human subject. The more recent publications are those of Thomsen (1929) and Laguna (1930). These authors described cases in which the receptor A of group AB was so poorly developed that its presence could only be demonstrated by the use of exceptionally strongly agglutinating sera. That a partial serum deficiency (agglutinin deficiency) occurs in individual animals of group O was shown by Hirszfeld and Przesmycki (1921), who could demonstrate the presence of anti B agglutinin of group O only in half the number of animals belonging to this group, whilst anti A could easily be demonstrated in all cases.

However, there is a further irregularity, viz., that the red cells of no two of these 23 horses (group AB) react in an identical manner with the sera of the 47 horses examined.

The red cells of three horses (20354, 20362 and 20418) are not agglutinated by the sera of any of the 47 horses used. The serum of 20354 agglutinates the red cells of 13, that of 20362 the red cells of 20 and that of 20418 the red cells of 21 out of the 47 horses used. These three horses (6·3 per cent.) would, therefore, appear to conform to the human group O requirements; their red cells give identical negative reactions against the sera of all the animals used, their red cells and sera do not react against one another, but it is nevertheless significant that their sera give different reactions to the red cells of the various horses examined.

The remaining 44 per cent. of horses gave such irregular reactions that definite grouping is not possible. It is, however, hoped at a later date to attempt the separation of the A and B factors by means of absorption tests.

Burghardt (1933) supplies these figures in percentages of the relative frequency of the different groups in horses according to various authors:—

	O.	*A.*	*B.*	*AB.*
Herszfeld & Przesmycki	9	70	15	—
Schermer	25	44·4	25	5·6
Thomoff	12	70	5	13
Burghardt	5·3	18·7	11·3	64·7

Without being able to give figures for groups A and B, the South African horses would seem to correspond in their reactions to those examined by Burghardt, viz. group O 6 per cent. and group AB 50 per cent. These differences in the relative frequency of the different groups are probably dependent on race differences.

SHOCK.

At the present time there is still considerable difference of opinion as to what actually causes transfusion shock. Coca (1909) believes that sudden death after injection of small quantities of red

cells from another species is due to mechanical occlusion of capillaries and arterioles in the pulmonary circulation. This mechanical occlusion is probably caused by agglutinated foreign red cells.

Ottenberg (1911) states that in the majority of cases intravascular agglutination does not occur. In the few cases where it does occur, it usually produces no symptoms. Occasionally, however, it may be the cause of untoward symptoms and even death.

Forssman (1925 and 1927) showed that peculiar cerebellar disturbances could be reproduced at will when making centripetal injections of haemolytic sera into the carotid arteries of guinea pigs (his so-called carotal complex). When the same haemolytic sera are injected intravenously anaphylactic symptoms are produced. When using 10 per cent. starch suspension in saline carotal symptoms are also produced. He concludes that these carotal symptoms are due to circulatory disturbances in certain portions of the brain (cerebellum, medulla oblongata) dependent on interference with the circulation due to the solid starch granules. How this effect is produced by the sheep rabbit haemolytic sera is not definitely known, but it is thought that such sera can bring about changes in the endothelial cells (Halber and Hirzfeld, quoted by Forssman, 1927) as a result of which the circulatory disturbance occurs. Forssman (1927) would further appear to believe that the haemolytic sera may in addition also injure nerve cells. In experiments with china ink, Forssman and Skoog (1925) were able to show that this substance does not protect animals against the so-called reverse anaphylaxis whereas such protection is afforded against the genuine anaphylaxis, as well as against the carotal symptoms due to centripetal injections of haemolytic sera in guinea pigs.

Lemke (1925) in reviewing the literature concerning fatal cases as a result of transfusion, refers in some detail to the case of Kuczynski, where the cause of death would seem to have been due to extensive thrombosis of the smaller arteries and capillaries of the lungs. He gives a detailed macro- and microscopic description of two fatal cases after transfusion. In the first case haemolysis developed in spite of the use of a compatible donor. In the second case there was no haemolysis. In both cases there were very extensive haemorrhages and severe necrosis of the liver. In the first haemolytic case there were also pronounced degenerative changes in the kidneys. He believes with other authors (Kusama, Lieber, Behne) quoted by him, that the dangers of transfusion are not completely eliminated when the factors of agglutination and haemolysis have been excluded. He bases the assumption of a toxic effect mainly on the haemorrhages and liver necroses which are present also in cases where haemolysis did not occur.

Lindau (1928) maintains that there is no evidence to show that agglutination is a factor in the reactions due to transfusion. He quotes Kusama, who found in animals which survived certain injections many more capillary thrombi, than in others which died and concluded that such a blockage of the capillaries could not be the direct cause of death. He further quotes Behne and Liebner (1921) who believe that agglutination is of minor importance in the

production of reactions, but is of great significance as an indicator of the presence of haemolysins. Lindau classifies the reactions after blood transfusion as (1) mild reactions, and (2) severe haemolytic reactions.

The symptoms of the *mild reactions* may occur within ½ to 1 hour after transfusion. These consist in rigors, transitory (vorübergehende) temperature, a sense of being unwell and urticaria. Although these reactions may now and again occur irrespective of the method of transfusion, they are often associated with the citrate method of transfusion (20-50 per cent.), whereas by direct transfusion mild reactions occur to the extent of only 5 per cent, (Beck 1928, Brines 1928 quoted by Lindau). It would further appear that the chances that such reactions will develop are greater the more the blood is handled and the longer it takes after withdrawal of the blood from the donor and its injection into the vessels of the recipient. Mild reactions occurring somewhat later after transfusion and often accompanied by urticaria are ascribed to foreign proteins in the plasma. These reactions are harmless, although they may cause considerable discomfort to the patient.

Severe haemolytic reactions. This is the type of reaction which is usually encountered when a transfusion is made from an incompatible donor. The symptoms are: By the time 50-100 c.c. of blood are transfused the patient may experience a sense of tightness and strain in the head and in the region of the heart. Somewhat later there are violent pains in the lumbar region. The face becomes flushed and cyanotic, the pulse 20-30 per minute, but later becomes small and increased in frequency. There is often loss of consciousness for some minutes. In 15-60 minutes there are rigors and the temperature rises. There is in nearly all cases haemoglobinuria, now and again icterus, sometimes involuntary defaecation and later even blood-stained evacuations. A patient with such symptoms may recover but more frequently there is a fatal termination in periods of time varying from minutes to hours after transfusion. Usually, however, death takes place after a week, sometimes even 10-15 days or longer after transfusion. In these cases the kidney is the seat of most important disturbances with oliguria, anuria and uraemia. The lesions of the kidney are of a degenerative nature (nephrosis). An acid reaction of the urine would seem to be particularly dangerous for the development of anuria. (The paper of Baker and Dolds quoted by Lindau and others was unfortunately not available to me). Haemorrhages are present in serous and mucous membranes as well as necrosis of the liver. He emphasises the similarity both anatomico-pathologically and clinically of blood transfusion shock and anaphylaxis and believes with Pfeiffer (quoted by him) that the lesions and symptoms in both cases are dependent on the disintegration of albuminous substances which are set free in the circulation in large amounts, causing injury particularly to the liver and the kidney.

Bayliss (1920) regards haemolysed blood as non-toxic and the disturbances resulting from transfusion from incompatible donors he ascribes to the action of foreign serum proteins analogous to that responsible for anaphylactic shock.

Borchardt and Tropp (1928) state that haemolysed blood in small doses produces no symptoms in dogs, but in greater doses may produce severe shock. They unfortunately do not state if the haemolysed blood was from a donor belonging to the same blood group or not. They conclude that (1) haematin is non-toxic, (2) globin may be responsible for the occasional shock which occurs in those diseases associated with severe destruction of red cells and (3) the stromata cause fever and rigors in these diseases.

Witts (1929) emphasises the necessity of having an alkaline urine in order to prevent anuria in cases of haemolysis which may occur as in the case described by him, where preliminary grouping and cross-agglutination tests had shown no incompatibility. In this connection it should be noted that Kolmer and Motomatsu Matsumoto (1920) in testing human erythrocytes against horse serum have found that haemolysins may be present and hemagglutinins absent and vice versa.

Wildegans (1930) discusses the relationship of agglutination and haemolysis. He states that there is a good deal of evidence that haemolysis may take place without any previous agglutination and suggests that in addition to agglutination greater attention should be paid to haemolysis than is being done at present in order to exclude as far as possible all the dangers of transfusion. This author also warns against the use of the universal donor for receivers suffering from anaemia. According to him the primary disturbances in fatal cases after transfusion are due to changes in the kidney, liver, heart and blood vessels. The oliguria and anuria are due to haemoglobin infarcts and occlusion of the urinary tubules by haemoglobin cylinders, in addition to parenchymatous degenerative changes. The haemorrhages in mucous and serous membranes are partly ascribed to co-existing toxic substances.

Already in 1913 Ottenberg and Kaliski emphasised the necessity of excluding both agglutination and haemolysis before transfusion. They also described erythrophagocytosis when incompatible blood is transfused.

Barrat and Yorke (1911) in making intravenous injections into rabbits of (*a*) haemolysed blood, (*b*) haemoglobin solution which was, as far as possible, completely freed from red cell stromata, and (*c*) stromata as far as possible completely freed from haemoglobin, concluded that the stromata is the more important and the haemoglobin the lesser factor in the production of disturbances. When large doses of haemoglobin are injected anuria may result. From the evidence available it is not clear if the disturbances are of the nature of anaphylaxis or whether poisonous substances which are not directly associated with the production of anaphylaxis are responsible. A number of other authors Zinsser (1923), Dold (1929), Scott (1931) emphasise the similarity of blood transfusion shock and anaphylaxis, but the exact relationship of the two conditions has not yet been definitely established.

As already stated a variable number of animals develop symptoms of shock both during or soon after transfusion of whole blood or the intravenous injection of fairly large amounts of horsesickness immune

serum. The symptoms occur in both horses and mules.* The first noticeable symptom is associated with respiratory disturbances. The respirations become more frequent and even laboured. The nostrils are dilated, the animal blowing with heaving flanks. It may rear and fall backwards or may stagger about until it drops. When this occurs, it lies on its side with marked abdominal breathing, the limbs rigidly extended or drawn up against the abdomen. Some such cases terminate fatally. The upper lip is frequently retracted like that of a snarling dog and sometimes the animal yawns and even neighs feebly. Defaecation is frequent and involuntary micturition may occur. It seems to be the general opinion of those veterinarians who have had practical experience of shock with horsesickness immunisation, that recovery takes place after micturition and defaecation has occurred. The animal may sweat profusely and in a proportion of cases urticarial swellings develop. When such swellings appear they are very small, but they may enlarge rapidly until they attain the size of walnuts. Some may coalesce to form a fairly extensive raised area. No information as to the presence or absence of a temperature reaction is available.

If the symptoms noted are due to blood group incompatibility, it should be possible to reproduce them almost at will. Experiments were accordingly undertaken with this end in view.

The experimental production of shock may be conveniently discussed under:—

I. Direct transfusion of whole blood using a donor with an incompatible blood group and a donor with a compatible blood group as control.

II. The intravenous injection of own transformed corpuscles. (Thomsen's haemagglutination phenomenon.)

III. The intravenous injection of haemolysed blood: (*a*) own haemolysed blood, (*b*) haemolysed blood from a compatible donor (belonging to the same blood group), and (*c*) haemolysed blood from an incompatible donor.

I. Results of Transfusion using Donor with Incompatible Blood Group.

Transfusion Experiment No. 1.

Horse 20234 was selected as donor to horses 20232 and 20346. Horse 20232 acted as control, as neither the red cells nor the sera of horses 20234 and 20232 react with one another. The red cells of 20234 were agglutinated by serum of 20346, but the serum of 20234 did not agglutinate the red cells of 20346. Transfusion was carried out for five minutes at the rate of 500 c.c. per minute. The effects of this on the number of respirations of the two horses is tabulated below:—

* A description of these symptoms was kindly given to me by Mr. B. v. d. Vyver, Government Veterinary Officer, Pretoria, and by Mr. W. Averre, Senior Lay Assistant of this Institute, both of whom have a very wide experience in horsesickness immunisation and hyperimmunisation.

TABLE 2.

	20232.	20346.
Number of respiration before transfusion.....	40	32
Five minutes after transfusion.....	—	48
Ten minutes after transfusion.....	—	51
Fifteen minutes after transfusion.....	44	53
Twenty minutes after transfusion.....	—	51
Thirty minutes after transfusion.....	40	40
Fifty minutes after transfusion.....	—	36

Horse 20232 did not react in any way as a result of transfusion. In horse 20346 the number of respirations became more frequent and the animal in addition made peculiar working movements with the lips, yawned, and defaecated at frequent intervals.

Transfusion Experiment No. 2.

The same experiment was repeated, using horse 20355 as donor, and 20357 and 20200 as receivers. Exactly the same group relationships were present here as in the previous case. Horse 20357 was the control. The results are recorded in Table 3.

TABLE 3.

	20357.	20200.
Number of respirations before transfusion.....	32	29
Ten minutes after transfusion.....	33	29
Twenty minutes after transfusion.....	30	26
Thirty minutes after transfusion.....	30	26

Here no disturbances in the respirations were noticed, but horse 20200 also worked the lips, yawned, defaecated at frequent intervals and urinated once.

In view of these negative results or at the most, the very mild disturbances exhibited by animals 20346 and 20200, the most favourable conditions for the development of shock were deliberately planned. In the previous cases, donors having red cells which are agglutinated by the serum of the recipients, were used. In the case now to be described, a donor was selected having, in addition, serum which agglutinated the red cells of the recipient. These group relationships existed between horses 20359 and 20415.

Transfusion Experiment No. 3.

Horse 20415 was used as donor. Transfusion at the rate of 500 c.c. per minute was carried out for eight minutes, approximately four litres of blood being transfused. The result of this on the respirations of horse 20359 are recorded in Table 4.

TABLE 4.

	Horse 20359.
Number of respirations before transfusion	26
Ten minutes after transfusion	58
Twenty minutes after transfusion	48
Sixty minutes after transfusion	13
Three hours after transfusion	17

Within three minutes of the commencement of transfusion, the animal started to blow, inspiration became prolonged and deep, with marked movement of the ribs. Soon this disappeared to reappear within three minutes' time. Within 10 minutes after transfusion was completed the number of respirations increased to 58 and the pulse to 60 per minute. The animal became very restless and pawed the ground. Defaecation was observed six minutes after transfusion was commenced and after completion of the operation, it took place at frequent intervals, but in small amounts. Even when the animal was not actually defaecating it was observed to frequently raise the tail in an apparent attempt to defaecate. Soon after the cessation of transfusion the animal broke out into a profuse rather cold sweat, large drops of sweat actually oozing from the skin at different places. When transferred to a loose-box and the head released, it lay down, looked back towards the abdomen, rolled occasionally and, in fact, exhibited symptoms characteristic of a mild attack of colic. The animal gradually became quieter and one hour after transfusion the respiration had decreased to 13 per minute, being slow and deep. Three hours later the respirations returned to more or less normal depth and were 17 in number. Four hours after transfusion the animal started to feed. Yawning was not noticed in this animal, and although urination did take place some considerable time after transfusion, the actual time of occurrence was not observed.

Conclusion.—A well-marked case of shock was produced by transfusing 4 litres of whole blood into a horse (20359) having serum which agglutinated the red cells, and red cells which were agglutinated by the serum, of the donor 20415.

About one year later this transfusion experiment (horse 20415 donor to 20359) was repeated as a demonstration to veterinary colleagues. This second transfusion took place 3½ months after horse 20359 had severe shock as a result of the intravenous injection of haemolysed blood from the same donor, details of which will be presented subsequently in this paper.

Within two minutes after the commencement of the transfusion (2 p.m.) the animal became unsteady. The transfusion was then interrupted for a minute or two and again continued. After this initial disturbance the transfusion did not seem to affect the animal in any way. The respirations remained normal and on this account 5 litres of blood were transfused instead of the four originally planned. After transfusion was completed, the animal became slightly uneasy, defaecated once, there was slight sweating and only

very slightly accelerated breathing. Two further sets of symptoms, which were not present at the first whole blood transfusion were, however, observed. No special importance was attached to these at the time, but later it was considered that they did have a special significance. These were: (1) fibrillary contractions of various muscles, and (2) the swaying of the body and sagging on one hind-quarter to be described later in cases where transformed corpuscles (Thomsen's phenomenon) were injected intravenously. The latter symptom was marked. An hour after the transfusion the symptoms observed were therefore interpreted as those of mild shock. Two hours after transfusion, the animal began to develop symptoms of severe shock. It was dull, obviously uncomfortable, and very unsteady. Defaecation was frequent, the faeces soft in consistency, but no urine was passed. Towards the evening the animal went down and, only with difficulty, could it be induced to get up. It consistently refused all food and water. It was given $\frac{1}{2}$ grain of arecoline hydrobromide subcutaneously, as a result of which a fair amount of liquid faeces was passed and profuse sweating took place. The next morning the animal supported itself against the walls of the stable. When forced to walk it would knock itself against any object in its way, as though blind. As a result of this there was injury of the tissues over the bony prominences of the face and the symptoms somewhat resembled those of acute liver atrophy described by Theiler (1918). An intravenous injection of 100 gm. of glucose in a litre of sterile saline was given that morning. At 2 p.m., i.e. 24 hours after transfusion, the animal was very weak and had apparently passed no urine. The tongue was hanging out and was markedly bruised and lacerated. The catheter was passed, but no urine could be obtained. An examination of the blood revealed a very marked haemoglobinaemia. At 3 p.m. the animal lay stretched out on its side in a state of collapse. As the chance of recovery was remote, the horse was shot and a post-mortem examination carried out. This was largely negative. There were extravasations particularly of the serosa of the intestines and to a less extent of the pleura. The kidneys were not enlarged but were paler in colour than normal. The liver contained a large amount of blood, the central veins were dilated, but no other macroscopic changes were recognised.

Microscopic Examination: Liver.—The central veins were distended with blood and a good deal of blood was also present between the cell rows. The stasis was so marked in places that it resembled to some extent the early stages of acute liver atrophy described by Theiler (1918). The liver cells showed degenerative changes. The cytoplasm was granular, many cells containing fat in the form of a fine granular dust, but occasionally cells were seen containing large fat droplets. There was a considerable amount of haemosiderin, not only in the reticulo-endothelial cells, but also in the liver cells. In addition to the haemosiderin a yellowish brown staining pigment, probably a non-iron-containing derivative of haemoglobin, was observed.

Kidney.—The renal vessels were markedly distended with blood. In some places the red cells were present in clumps of varying size, each clump consisting of 5 to 20 cells. Actual haemorrhages were

irregularly present throughout the substance of the kidney, but they were especially well marked in the medulla. A certain amount of haemoglobin was present in Bowman's capsule and in some of the renal tubules, but haemoglobin cylinders, as such, were not well formed. Only a small amount of fat was observed. A considerable amount of haemosiderin granules were scattered throughout the kidney, as well as granules which did not stain for iron. These were, as in the case of the liver, probably non-iron-containing derivatives of haemoglobin.

The spleen contained a large amount of blood and haemorrhages were present. The lungs showed hyperaemia, emphysema and haemorrhages with blood in the pulmonary alveoli.

Conclusion.—The cause of death may be ascribed to severe haemolytic shock caused by the transfusion of 5 litres of blood from a donor whose red cells were agglutinated by the serum of the receiver and whose serum agglutinated the red cells of the receiver. To what extent agglutination in vivo may have been a contributory factor in the production of the fatal shock is not known.

Witts (1929) analysing transfusion fatalities (from figures supplied by Oliver for the blood transfusion service of the British Red Cross Society) stated that not a few deaths have resulted when practising transfusion with the universal donor (group O). In view of this statement an attempt was made to estimate the relative danger of transfusing blood having (*a*) an agglutinating serum (its red cells not being agglutinated by the serum of the receiver), and (*b*) red cells which are agglutinated by the serum of the receiver (its serum not agglutinating the red cells of the receiver).

Transfusion Experiment No. 4.

These group relationships existed between horse 20415 (donor) and horses 20366 and 20208 (receivers). Four litres of blood were transfused into each of the two horses. In neither animal was shock observed. This failure may be ascribed to the weakness of the agglutinin content of the serum of the horses used, leaving out of consideration the possibility that haemolysis may be the essential factor in the production of shock when blood is transfused from an incompatible donor. The shock which was artificially produced by transfusing from 20415 into 20359, as above described, was then presumably due to the fact that cross-agglutination was present. To control this point, a further transfusion experiment was planned.

Transfusion Experiment No. 5.

5. (*a*) Four litres of blood were transfused from horse 20258 into horse 20368. The object of this was to see if serum from a donor agglutinating the red cells of the recipient, would cause shock when the red cells of the donor are not agglutinated by the serum of the recipient. The transfusion was completed in 8 minutes, the animal, although of a somewhat nervous temperament, showing no marked disturbance. The respirations, 44 per minute before transfusion, increased to 60 per minute within 15 minutes, after transfusion. During this time the animal became restless, pawed the

ground and broke out into a sweat. Defaecation was frequent and later developed into a mild diarrhoea. These symptoms gradually improved and after some hours the horse was more or less normal again.

Conclusion.—Well-defined shock was produced when transfusing blood, the serum of which agglutinated the red cells of the recipient, even though the red cells of the donor were not agglutinated by the serum of the receiver. According to these results, it would appear that the use of the universal donor is contra-indicated, especially in the human subject where the agglutinins are so much more strongly developed. Lindau (1928) also warns against the use of the universal donor in the human subject. In this connection it is worthy of note that Lemke (1925) states that it is not possible to completely exclude the dangers of haemolysis even with previous precise serological tests.

5. (*b*) 3·5 Litres of blood were transfused from horse 20390 into 20271. The object of this was to see if transfused red cells which are agglutinated by the serum of the receiver, will produce shock, when the serum of the donor does not agglutinate the red cells of the receiver. After three minutes transfusion horse 20271 started blowing; this soon passed off, but almost immediately reappeared. After transfusing for 6 minutes, the animal became very restless and finally became so excited that the trochar in the jugular vein was jerked loose and the transfusion of 4 litres of blood could not be completed. The animal's respirations increased from the normal 30 per minute to 72 immediately after transfusion was completed; in 15 minutes the rate was 60 and 40 minutes after transfusion 42 per minute. The animal was restless, pawed the ground, defaecated frequently, but did not sweat at all. After a few hours the animal seemed to be quite normal again.

Conclusion.—Well-defined shock of a mild degree occurred when blood was transfused from a donor the red cells of which were agglutinated by the serum of the recipient, but the serum of which did not agglutinate the red cells of the receiver.

Transfusion Experiment 5 (c) *Control.*

The control animal 20367 was transfused for 8 minutes (4 litres) from the same donor 20390. Neither the red cells nor the serum of either of these animals reacted with that of the other. Horse 20367 at no time showed uneasiness or ill-effects from the transfusion. The day was hot and, in spite of the addition of 4 litres of blood to its circulation, no sweating occurred. On theoretical grounds sweating might be expected, but actual experience at these laboratories has shown that as much as 10 litres of blood may be transfused at one time without any untoward symptoms being produced. By that time the donor may already show respiratory distress owing to the great loss of blood, and circulatory disturbances from the decrease in blood volume and blood pressure.

Discussion.—Although well-defined shock was produced in horse 20368 [Transfusion Experiment 5 (*a*)] (agglutinating serum of donor) and an apparently milder degree of shock in horse 20271

[Transfusion Experiment 5 (*b*) agglutinated red cells of donor], it is noteworthy that horse 20368 was a small, rather nervous animal, and horse 20271 a much larger and more phlegmatic type of animal. It seems almost certain that size influences the degree of shock, which will be greater in the smaller animal, the volume of transfused blood being constant.

Furthermore, only 3½ litres of blood could be transfused into horse 20271 as against the 4 litres into 20368. To what extent a highly-strung nervous animal may be more susceptible to shock than a phlegmatic one, is not known. In the circumstances, one cannot compare the degree of shock in the two animals, and a conclusion that a transfusion from a donor whose serum agglutinates the red cells of the receiver (its red cells not being agglutinated by the serum of the receiver), is more dangerous in producing shock, than a transfusion from a donor, the red cells of which are agglutinated by the serum of the receiver (its serum not agglutinating the red cells of the receiver) would not be justified.

II. Thomsen's Haemagglutination Phenomenon in Relation to Transfusion.

To further determine the part played by agglutinated red cells in the production of shock use was made of red cells which were subjected to the action of bacteria of the M. and J. bacillus type, described by Friedenreich (1928 and 1930). Thomsen (1927) showed that human red cells may, on standing for 12 hours or more, become so changed that they may be agglutinated by any serum, including the serum of the individual from which the red cells were obtained. In 1928 and 1930 Friedenreich isolated two organisms which he named the bacillus " M " and the bacillus " J ". These organisms in pure culture were capable of transforming red cells in this manner. Further he showed that bacteria-free filtrates could similarly transform red cells. The same phenomenon was observed at these laboratories when working with equine red cells which were collected some days previously. From this blood Mason (1933) isolated in pure culture an organism similar to Friedenreich's J. bacillus. With this organism and filtrates prepared by Mason, equine corpuscles were readily transformed so that agglutination by their own sera was produced.

When studying transfusion shock Friedenreich (1928) made use of such transformed corpuscles in transfusion experiments in guinea-pigs. The transformed (own) corpuscles were injected intravenously into guinea-pigs. The symptoms which developed were at that time interpreted by Friedenreich as analogous to incompatibility transfusion shock of the human subject, but when he repeated these experiments in 1930 and injected the (own) transformed corpuscles intravenously into guinea-pigs, shock occurred in only four out of the 18 animals used. The other 14 guinea-pigs, in addition apparently to the controls, showed pronounced anaphylactoid symptoms, which were, however, mild in comparison with the severe shock observed in the four. Haemoglobinaemia was present in all these four cases (strong in three and moderate in 1). After a critical survey of the

whole question he concluded that transfusion shock produced by the intravenous injection into guinea-pigs of their own transformed corpuscles, is essentially due to intravital haemolysis. This question of haemolysis in relation to shock will be more fully discussed later when results of injecting haemolysed blood intravenously into horses are presented.

Experiments with Equine Transformed Corpuscles.

Experiment No. 1.

From horse 20290 three litres were bled into each of two flasks A and B, containing sufficient sodium citrate to prevent coagulation. After sedimentation of the red cells it was intended to remove the supernatant plasma containing citrate, replace this by normal saline and add to the one flask a culture of the transforming organism and keep the other flask as a control. Sedimentation was, however, very poor, and only a small amount of plasma was removed in each case. Sedimentation was better in flask A than in B. After replacing the supernatant fluid with sterile normal saline, both flasks were allowed to stand for a further 12 hours, when the supernatant fluid was again syphoned off and replaced by saline as before. At 4 p.m., on 21st October, 1932, a broth culture of the transforming organism was added to the red cell saline suspension in flask A. After allowing to stand for 36 hours at room temperature (summer time) $2\frac{1}{2}$ litres of the transformed red cell suspension were injected intravenously into the same animal 20290. It may be added that the transformed red cells were strongly agglutinated *in vitro* by their own serum.

Effects of Intravenous Injection of Transformed Red Cells.

Soon after injection was commenced the animal began to blow and defaecated. It became uneasy and restless, but before the injection was completed the respiratory disturbances had more or less disappeared. On the completion of the injection the animal continued to be restless, defaecated at frequent intervals, the faeces becoming progressively softer in consistency until eventually there was actual diarrhoea. The horse moved the lips, yawned and occasionally made a peculiar crouching movement, when the whole body would be lowered (depressed) with a hollow back. At other times it would sway violently towards one side, the hindquarter of that side dropping a good deal. It was that kind of movement an animal would make when struck smartly on the loins and would therefore appear to be analogous to the acute pain experienced by humons in transfusion shock.

The respirations before injection were 30 per minute.

The respirations 10 minutes after injection were 24 per minute.

The respirations 1 hour after injection were 22 per minute.

The respirations $1\frac{1}{2}$ hours after injection were 22 per minute.

The respirations 7 hours after injection were 50 per minute.

Six hours after injection the temperature increased to 106° F. At this time the respirations were not markedly accelerated, but were of a peculiar jerky type and much deeper than normal. The

animal gradually became weaker on its legs and 7 hours after injection could no longer maintain the standing position. The respirations during this time were 50 and the pulse 40 per minute. The animal died within 12 hours after injection. During the last three hours it was lying stretched out on its side. Sweating was observed only during the last four hours before death. The horse died at 9.30 p.m., on 2nd November, 1932, and a post-mortem examination was made at 7 a.m. the next morning. The outstanding feature revealed by this examination was the presence of numerous petechiae, ecchymoses and even extravasations throughout most of the organs. These haemorrhages were particularly numerous in the visceral pleura over the dorsal portion of the coastal surfaces of both lungs. Over these areas the haemorrhages had become confluent and produced a uniform reddish discoloration of the surfaces of the lungs. Haemorrhages were also well marked in the left endocardium and in the mucous membrane of the main bronchi. A small amount of viscid and opaque urine was present in the bladder, but there was no evidence of haemoglobinuria. That haemoglobinaemia was present cannot be stated with certainty. The spleen measured 60 by 23 by 4 cm., and could not be regarded as being definitely enlarged. There were quite a number of haemorrhages on the capsule of the spleen. There was a large amount of cerebro-spinal fluid. The blood vessels of the brain appeared to be injected. On section dark red spots were seen to be irregularly distributed throughout the substance of the brain.

Anatomical Pathological Diagnosis.

Petechiae, ecchymoses and extravasations particularly in the serous membranes and to a lesser extent in the various organs of the body; hyperaemia and slight oedema of the lungs, degenerative changes in the myocardium, liver and kidneys.

Aetiological Diagnosis.

Shock, due to the intravenous injection of 2½ litres of a saline suspension of its own red cells, which were transformed by the action of a " J "-like bacillus (Friedenreich) so that the red cells were agglutinated by their own serum.

Microscopic Examination.

Kidney.—There was marked hyperaemia, particularly of the vessels of the medulla. In places there were collections of red cells, throughout the substance of the kidney, and the impression was obtained that some capillaries were distended with clumps of red cells. There were some tubules which contained haemoglobin cylinders, but these were present only to a moderate degree. Only very little haemosiderin was demonstrable in the kidney. The cytoplasm of the tubular cells was granular and with Sudan III, showed the presence of fat in the form of a very fine granular dust.

Liver.—The central veins were distended with blood. In places there was a good deal of stasis around the central veins, which resulted in atrophy of the liver cells. In some cases actual

haemorrhages were present with more or less complete destruction of the liver tissue. The liver cells themselves were involved in fatty changes, the fat being chiefly present in the form of a very fine granular dust, but some cells contained larger fat droplets. There was a considerable number of cells which did not contain any nuclei at all. Others had nuclei in varying stages of degeneration showing pycnosis, etc.

Spleen.—Sections stained for iron with Berliner Blue revealed the presence of numerous large cells loaded with blue pigment granules. When this section was compared with a similarly stained section of a horse spleen where no blood destruction had occurred, one could readily recognise even with the naked eye, the much greater preponderance of iron-staining material. This would, in spite of the absence of haemoglobinuria and of the absence of definite evidence of haemoglobinaemia, justify the conclusion that a good deal of blood destruction had taken place in this animal. It is unlikely that the presence of the transformed red cells in the circulation would initiate a perverted function of the body to destroy its own normal red cells. The animal was apparently healthy when placed in the experiment and, as far as is known, was not suffering from any blood disease. It is, therefore, practically certain that the increased iron pigmentation in the spleen was derived from the destruction of the transformed red cells that were introduced.

Control.

It was intended to use the blood in flask B as a control. The blood in this flask was dealt with in exactly the same way as that in flask A but instead of adding the broth culture of the J bacillus 36 hours before intravenous injection, this was to have been added immediately before the injection. On the day on which the injection was to have been made the blood in this flask was haemolysed and was on that account not used.

Experiment No. 2.

In view of the well-defined shock produced with transformed red cells in the case of horse 20290, it was decided to repeat the experiment and include at the same time suitable controls.

Three litres of citrated blood were obtained from horse 20318. After sedimentation of the red cells was complete, the plasma was removed and replaced with sterile normal saline. To this suspension of red cells a culture of the transforming organism was added. As some particles of fibrin were present in the suspension, it was filtered through sterile muslin and two litres were injected intravenously into the same animal, 24 hours after the culture of the transforming organism had been added.

Effects of Intravenous Injection of Transformed Cells.

Soon after the injection was commenced the animal started to blow. The respirations became laboured and just as the injection was completed, the animal swayed and fell down. The respirations now were slow and deep. The animal lay stretched out on its side

and continued to breathe with great difficulty. Whilst still down the respirations suddenly increased in number to 74 per minute as against 13 before injection. Within 10 minutes improvement took place and the animal was able to rise. It now developed symptoms similar to those seen in horse 20290, particularly in so far as swaying of the body and crouching movements were concerned. Defaecation was frequent, but at no time was there diarrhoea as in the case of horse 20290. The respirations became less frequent but remained deeper than normal for several hours after injection. Seven hours after injection the condition of the animal had markedly improved, and it commenced feeding again. Twenty hours after injection the animal was feeding normally, but the respirations continued to be somewhat deeper than normal. There was no sweating at any time and colicky symptoms were not observed.

(*b*) *Controls.*—(1) Horse 20367 was bled on the same day as horse 20318 and the blood treated in exactly the same way except that no culture was added to it. After standing for 24 hours this red cell saline suspension was injected intravenously into the same animal (20357) and at the same time as the injection into horse 20318 was made, with entirely negative results.

(2) Horse 20293 was bled and after coagulation was complete the serum was collected aseptically and a culture of the bacillus equal in amount to that used in the case of horse 20318 was added. To the serum was further added sterile normal saline equal in volume to that of the fibrin and red cells from which it was removed. After this serum saline mixture was exposed to the action of the transforming organism for 24 hours, it was injected intravenously into the animal from which the serum was originally obtained, without the animal developing any symptoms whatsoever.

Conclusion.—(1) Well-defined shock was produced in two horses by the intravenous injection of their own transformed corpuscles. It is perhaps not without significance that in the one case 2 (*a*) in which recovery took place, the red cells were only subjected to the action of the transforming organism for 24 hours (as against 36 hours in the fatal case) and only 2 litres of blood were used.

(2) In neither of the controls were any disturbances observed. Transformation of the red cells was therefore responsible for the production of the pronounced shock. The only known difference between the transformed and normal corpuscles consisted in agglutinability in vitro. To what extent the transforming organisms may in addition have changed the red cells biologically or chemically, is not known. Fragility tests carried out by Friedenreich (1930) showed that in this respect the transformed corpuscles do not differ in any way from normal corpuscles. It is, however, doubtful if such in vitro tests are a true indication of what will take place in vivo and if one can judge from the lesions in the kidneys and spleen, one must conclude that a certain amount of haemoglobinaemia was present, but as far as could be made out this was of a moderate degree.

In view of the harmlessness of own haemolysed blood or even haemolysed blood from a compatible donor, as will be shown in subsequent experiments in this paper, it is difficult to understand, even assuming that appreciable haemolysis did take place, that haemolysis is responsible for the shock. If that is the case, one must assume that the haemolysed blood or perhaps more particularly the haemoglobin molecule, had become transformed into a toxic principle. Until more definite evidence in this connection is available, it would seem that the mechanical factor (agglutination) cannot be excluded as the cause of shock when transformed corpuscles are injected intravenously.

III. Experiments with Haemolysed Blood.

In view of the absence of definite information as to the toxicity or otherwise of haemolysed blood, it was necessary to find out what the effect would be of injecting an animal intravenously with its own haemolysed blood.

Experiment No. 1.—Own Haemolysed Blood.

(*a*) Four litres of citrated blood were obtained from horse 20331. After sedimentation was complete (± 20 hours after bleeding) the supernatant plasma was removed and replaced with an equal volume of sterile distilled water, which caused complete haemolysis. This haemolysed solution was warmed to ± 37° C. and injected intravenously into the same animal about 24 hours after the original bleeding. The animal was not in any way disturbed during the injection. Subsequently it developed haemoglobinuria and icterus, but otherwise appeared to be normal. This method of preparing haemolysed blood was applied in all subsequent experiments, and it is of importance to emphasize that all the solid elements in the blood, viz., leucocytes, thrombocytes and red cell stromata, were in this way injected intravenously, the only substance removed being the citrated plasma.

(*b*) The same test was repeated with horse 20491. The animal defaecated once during the injection, the respirations became somewhat more frequent, but the animal did not appear to be unduly disturbed. After the injection was completed, the skin became hot and very slight sweating took place. The symptoms were so mild that they could scarcely be described as those of even mild shock. Later on haemoglobinuria and icterus appeared.

Experiment No. 2.—Effects of Haemolysed Blood from a Compatible Donor.

Six litres of haemolysed blood were prepared from horse 20234. This was divided into two equal parts. Three litres were injected into horse 20373 and three litres into horse 20368. No incompatibility was revealed when the bloods of these two horses were tested against that of the donor.

Except that the temperature rose from 100·2° (pre-injection) to 101·6° F., one hour after injection, no symptoms whatever were observed in horse 20368.

Horse 20373 developed peculiar crouching movements during and after injection. The respirations became frequent and defaecation took place at short intervals. The pre-injection temperature was 100·4 and post-injection temperature 100·8. Within half an hour after injection the animal fed normally. There was no sweating and no shock.

Conclusion.—Very large amounts (4 litres) of an animal's own haemolysed blood was found to be non-toxic in two horses tested and large doses (3 litres) of haemolysed blood from a compatible donor were found to be non-toxic in two horses tested.

Experiment No. 3.—Effects of Haemolysed Blood from Incompatible Donors.

(*a*) Four litres of haemolysed blood were prepared from horse 20415 and injected intravenously into horse 20359. Previously 20359 had developed severe shock when it received 4 litres of whole blood from 20415 by transfusion. There was cross-agglutination between these two horses. The animal started blowing during the injection, defaecated at frequent intervals, became very unsteady on its legs, and appeared likely to fall at any moment. Before injection was completed, it began to sweat, particularly around the base of the ears. After the injection was completed it broke out into a severe general sweat and the temperature increased from 99·4 to 100·6° F. The skin, which was hot whilst sweating was taking place, was again quite cool one hour after the injection had been completed. There was marked haemoglobinuria but no icterus within a day after injection.

(*b*) To further test out if haemolysis is a factor in the production of shock the same animals used in transfusion experiment No. 2 were employed, but on this occasion intravenous injections of haemolysed blood were given. Horse 20355 was the donor and 20357 the compatible receiver and 20200 the incompatible receiver. Three litres of haemolysed blood were injected intravenously into each animal. The control 20357 stood the full injection very well. Immediately after the injection was completed it was slightly restless and pawed the ground for a short time and respirations became slightly accelerated. The pre-injection temperature of 100·4° F. increased to 101·5° F., but apart from these slight disturbances no evidence of shock was manifested. In horse 20200, very soon after injection was commenced, the repirations increased in number and the animal became unsteady on its legs. By the time 200 c.c. were injected the horse fell down with such a marked respiratory dyspnoea, that it even opened its mouth to breathe. Whilst down, the animal was observed to bleed from the nose and sometimes lay stretched out on its side. The skin was hot and profuse sweating took place. Large drops of sweat formed around the base of the ears and on other parts of the skin. The animal gradually improved, and within half an hour was able to rise. An hour after injection was commenced the temperature had returned to the pre-injection level, viz. 99·6° F. Unfortunately, the temperature was not taken during the time the animal was showing severe symptoms of shock.

Conclusion.—Three litres of haemolysed blood from a compatible donor (20355) did not produce shock in horse 20357. Four litres of haemolysed blood from an incompatible donor (20415) produced severe shock in horse 20359. Two hundred c.c. of haemolysed blood from an incompatible donor (20355) produced very severe shock in horse 20200.

Discussion.—That only mild shock was produced when three litres of whole blood were transfused (Horse 20200) (transfusion experiment No. 2) is therefore presumably due to the absence of haemolysis even though well-marked iso-agglutination was demonstrable *in vitro.* (Serum of horse 20200 very strongly agglutinated the red cells of 20355). This would tend to confirm the opinion that haemolysis is the essential factor in the production of severe shock. These results further show that large amounts of own haemolysed blood and large amounts of haemolysed blood from a compatible donor, can be tolerated with very little, if any, disturbance, whereas a relatively small amount of haemolysed blood from an animal with an incompatible blood group may produce grave and alarming symptoms of shock.

(*c*) *Can Immunity be Produced against the Factor which Determines Incompatability of Haemolysed Blood?*

At this stage an attempt was made to immunise horse 20200 against haemolysed blood of 20355. Five hundred (500) c.c. of haemolysed blood were prepared from horse 20355 on 10th July, 1933, and 50 c.c. were injected subcutaneously into 20200 on 11th July, 1933, without any harmful effects. The temperature before injection was 99 and after injection 98·6. This was repeated on 15th July, 1933, with the same results. Temperature before and after injection being the same. On 18th March, 1933, 75 c.c. of haemolysed blood were injected subcutaneously, and on 21st 100 c.c., in both cases without producing any reaction whatsoever. On 27th March, 1933, the immunity was tested by injecting haemolysed blood from 20355 intravenously into 20200. Soon after the injection was commenced the animal started blowing, defaecated frequently and developed clonic contractions of the muscles on the side of the chest. When 400 c.c. had been injected the animal was on the point of collapse and the injection was discontinued. The animal reared and fell down panting for breath and it seemed as if a fatal termination was imminent. After spraying with cold water, the acute symptoms disappeared. The animal lay stretched out on its side, with laboured breathing, the skin felt hot and sweating undoubtedly occurred, but could not be seen easily on account of the previous application of cold water. The temperature before injection was 99° F. and 20 minutes after injection 100·6° F. The symptoms gradually improved and within 1½ hours after the injection the animal could again rise.

Conclusion.—Severe shock was produced in an animal by the intravenous injection of 400 c.c. of haemolysed blood from an incompatible donor in spite of having received, during the previous 17 days, repeated subcutaneous injections of the same haemolysed blood in increasing doses. This animal therefore did not develop any

appreciable immunity as a result of this treatment. It is obviously out of the question to draw general conclusions from the results obtained on one animal. However, it is of interest to note that Friedenreich (1930) would appear to have failed to immunise guinea-pigs against transformed corpuscles, but on the other hand Forssman and Skoog (1925) maintain that anti-anaphylaxis is easily obtained against the so-called reverse anaphylaxis by the injection of sub-lethal doses of the haemolytic serum, but the protection so afforded is only against a little more than 1 M.L.D. and never against 2 M.L.D. In the above case alarming symptoms developed after the injection of 400 c.c. of haemolysed blood from 20355, whereas previously the injection had to be discontinued after 200 c.c. had been administered. It is very doubtful if this bigger dose is of special significance, as this animal (20355) was at that time repeatedly bled 3-4 litres at a time, and it is believed that the decrease in the number of the red cells brought about in that manner, was responsible for the bigger dose, rather than an immunity which had developed.

Experiment No. 4.

In order to obtain further information of the rôle played by haemolysis in shock, transfusion experiment No. 5 was repeated, using on this occasion haemolysed blood intravenously injected instead of whole blood directly transfused.

The horses used in transfusion experiment No. 5 were 20390 donor to 20367 (compatible control) and 20271 (incompatible receiver) and 20258 donor to 20368 (serum of 20258 agglutinated the red cells of 20368, but the red cells of 20258 were not agglutinated by the serum of 20368). Horses 20390 and 20367 were, unfortunately, not available for this experiment and horse 20355 was substituted for 20390 and 20232 for 20367.

Three litres of haemolysed blood from horse 20355 were injected intravenously into (*a*) 20232 (compatible control), (*b*) 3 litres into 20271 (incompatible receiver) and (*c*) 3 litres of haemolysed blood from 20258 were injected into 20368.

Results obtained: (*a*) Control, during the injection the animal was somewhat uneasy and defaecated once. Within a few minutes after the injection was commenced the animal started to blow, but this soon passed off again. After the injection was completed, the animal still showed slight uneasiness, occasionally pawed the ground and now and again lifted either a front or a hind leg. The temperature increased from 99·6° F. (pre-injection) to 101° F. twenty minutes after injection and fell to 100° F. one hour after injection. The pre-injection respirations of 28 per minute increased to 60 per minute 10 minutes after injection. The skin remained cool throughout and no sweating took place.

Conclusion.—There was slight disturbance, but no shock, when 3 litres of haemolysed blood were injected intravenously into a compatible receiver.

(*b*) Incompatible receiver 20271—soon after the injection was commenced the animal started to blow. It became very uneasy and respiratory dyspnoea developed. There was well-marked trembling (clonic contractions) of the muscles of both hind limbs. The horse strained severely, but did not defaecate. (The animal defaecated normally just before the injection was commenced.) The animal seemed to be on the point of collapse when the flow of haemolysed blood was, by mistake, interrupted for ½-1 minute. The symptoms immediately improved and the animal was then given the remainder of the three litres of haemolysed blood. The pre-injection temperature was 101° F. Twenty minutes after injection the temperature was 101° F. and one hour after injection 100·6° F. The respirations increased from 28 (pre-injection) to 48 (post-injection). After injection the animal was dull and stood with hanging head; slight bleeding from the nose took place, but only moderate sweating, especially between the hind legs, occurred.

Conclusion.—The disturbances observed were those of well-defined but mild shock, but nevertheless of a more severe degree than that noted when a direct transfusion of whole blood was made. However, the severity of the shock was much less than that produced by incompatible haemolysed blood in 20359 and 20200.

(*c*) Horse 20368 stood the full intravenous dose of 3 litres of haemolysed blood from 20258 very well. The animal, although of a nervous temperament, merely showed, and that only occasionally, very slight restlessness during the injection. The respirations became slightly accelerated but could not even be described as those of a simple dyspnoea. No defaecation occurred during the injection and when the animal was brought to its stable, some 10 minutes after the injection was completed, it immediately started feeding. Its temperature increased from 101·2° F. to 101·6° F. twenty minutes after the injection was completed. No sweating took place. Therefore, except for slightly accelerated respirations, no disturbances whatsover occurred as a result of the intravenous injection of the haemolysed blood. This is really what one would expect, as the in vitro test showed that the red cells of horse 20258 were not agglutinated by the serum of 20368. Therefore one may presume that it was the serum of 20258 which produced the shock when whole blood was transfused. This was accordingly tested out. Three litres of blood were drawn from horse 20258. After coagulation the serum was collected and injected intravenously into horse 20368. There was slight restlessness, pawing of the ground, but no sweating. One is not justified in interpreting these slight disturbances as those of shock. What then was responsible for the shock when a transfusion of whole blood was made [transfusion experiment 5 (*c*)]? The only substance which was injected then and not injected when haemolysed blood and serum were separately used is fibrinogen. In view of the many transfusions which are made with whole blood without producing disturbances, it is obvious that fibrinogen cannot be incriminated as the toxic factor, especially in view of the fact that red cells alone and serum alone can produce shock. Doan (1927), however, quotes Genou (1901), Brodie (1901) and Starlinger (1925) who believe that blood plasma and blood serum may have different

biological properties. There is the further possibility that the animal may in the meantime have developed an immunity as is suggested by Forssman and Skoog in the case of reverse anaphylaxis, particularly in view of the fact that incompatibility as shown by *in vitro* tests was due to the serum and not to the red cells. Unfortunately there were not sufficient available horses showing this group relationship to test this out more fully. If this is the case, it would seem to be possible to immunise individuals against incompatibility of the so-called universal donor. It should, however, be noted that in this particular case leucocytic incompatibility was not controlled and that factor may possibly be responsible for the shock in the original whole blood transfusion as suggested by Sabin (1923) quoted by Doan (1927).

Experiment No. 5.

Three litres of haemolysed blood were prepared in the usual way from horse 20418 and injected on the same day into horse 20258. The red cells of 20418 were agglutinated by the serum of 20258, but the serum of 20418 did not agglutinate the red cells of 20258.

Horse 20258 stood the intravenous injection of 500-800 c.c. of the haemolysed blood very well without showing any symptoms. He then suddenly started to blow heavily and soon after that reared. The injection was discontinued, but the animal fell in an awkward position in the bleeding crush, from which it was dragged free in a couple of minutes. An attempt to stimulate it by applications of cold water to the skin, and artificial respiration was without avail. A post-mortem examination conducted within an hour after death revealed slight oedema of the lungs, extravasations in the visceral pleura and extravasations in the smaller bronchi. The mucous membranes were pale throughout. No histological examination was made.

Conclusion.—Severe shock with a fatal termination was produced within 10 minutes after the commencement of the intravenous administration of 500-800 c.c. of haemolysed blood from an incompatible donor.

What is the Factor which Determines Incompatibility of Haemolysed Blood?

This question will not be completely answered in this paper. Reference has already been made to the various views held by different authors in this connection, but before presenting details of an experiment with haemoglobin, it will be as well to summarise the deductions that can be made from the foregoing experiments with haemolysed blood. These results indicate that there is considerable experimental evidence to show that (1) own haemolysed blood does not produce shock; (2) haemolysed blood from a donor whose red cells are not agglutinated, and particularly not haemolysed by the serum of the recipient, does not produce shock, although the temperature reactions which may occur when own haemolysed blood as well as when haemolysed blood from a compatible donor is used may possibly be due to the action of the stromata as suggested

by Borchardt and Tropp (1928), already referred to, and (3) haemolysed blood from a donor whose red cells are agglutinated or haemolysed by the serum of the receiver produces shock, which may in some cases be rapidly fatal even when relatively small doses are injected. It seems to be generally accepted that the chances of producing transfusion shock are greater when the red cells of the donor are agglutinated or haemolysed by the serum of the receiver and the greatest shock-producing factor appears to be intimately associated with the red cells. According to Krumbhaar (1928) eight to nine-tenths of the erythrocyte consists of haemoglobin in solution, 4 per cent. of which consists of haematin and 96 per cent. of the protein globin. The erythrocyte further contains a stroma about the composition of which very little seems to be known, but Krumbhaar quotes Beumer and Burger (1923) who obtained 22·6 gm. of dry stroma per Kg. of red corpuscles. Further, the erythrocytes contain salts of Na, K, Mg, Ca, Cl and P, and in addition glucose, ferments, ± 2 per cent. of other proteins; and traces of other substances. If it be assumed that the incompatibility factor is contained in the erythrocyte, one may, after a complete analysis and having obtained each substance in the pure state, assign the role played by each factor in shock and other disturbances, particularly if one knows not only the entity or entities producing positive reactions, but also those in which no reaction is obtained. Strong confirmatory experimental evidence that the red cell as a whole is not necessarily concerned in the production of severe disturbances such as shock, is furnished by the experiments on horse 20200. It will be remembered that 4 litres of whole blood transfused from an incompatible donor (20355) into horse 20200 produced only very slight disturbances, but no shock. When 200-300 c.c. of haemolysed blood from this donor were injected intravenously very severe shock was produced.

When red cells are laked haemoglobin and other soluble substances will dissolve in the plasma. The insoluble stroma may, as already pointed out, be partially responsible for disturbances such as fever, but unless the stroma from cells of the incompatible donor is different structurally, physiologically, or toxicologically from that of the compatible donor, it cannot play any part in the production of severe shock, as proved by the experiments already detailed with own haemolysed blood and haemolysed blood from compatible donors. Indeed, the same is true for haemoglobin, and in view of the statement made by Borchardt and Tropp that globin is toxic an attempt was made to obtain haemoglobin* in a pure state from horse 20355, in order to compare the effects produced by it on horse 20200, with those already described when whole blood and haemolysed blood (including stroma) were used.

Three litres of blood were obtained from horse 20355. After complete sedimentation the red cells were removed from the supernatant coagulum and serum. The mass of red cells was subjected to dialysis in order to prepare crystalline haemoglobin according to the method of Dudley and Evans (1921). After dialysis the

* (Dr. Rimington, Empire Marketing Board Fellow, working at this Institute, kindly undertook the preparation of the haemoglobin.)

haemolysed mass was spun until complete precipitation of stromata, etc., was obtained. The supernatant fluid was carefully collected and regarded as a pure solution of haemoglobin. After bubbling through oxygen haemoglobin crystals were recognised microscopically. This concentrated mass of haemoglobin, partly in crystalline form, was diluted with sterile saline to a volume equal to that which would have been obtained if the red cells of horse 20355 were haemolysed in their own plasma. The diluted haemoglobin solution was filtered through ordinary filter paper, heated to approximately 37° C., and 300 c.c. were injected intravenously into horse 20200.

Effects of the Intravenous Injection of Haemoglobin from an Incompatible Donor.

During the injection of the 300 c.c. of haemoglobin no disturbances were observed, but within two minutes of the completion of the injection the animal became restless and unsteady, particularly in the hindquarters. It walked with a staggering gait and then collapsed with a marked inspiratory dyspnoea. Cold water was immediately applied to the whole body with a hose and later injections of atropin and adrenalin were given. After that the animal improved somewhat, but could not get up. It was carried to a loose-box, but soon after it became progressively worse, death supervening 4-5 hours after it had received the haemoglobin injection. A post-mortem examination was made within an hour after death and the following anatomical pathological diagnosis made: petechiae, ecchymoses and extravasations of the:—parietal and visceral, pleura, epi- and endo-cardium and the serosa of the intestines. Haemorrhages throughout the substance of the kidneys, large haemorrhages in the spleen, marked stasis of the liver and very marked emphysema of the lungs. The haemorrhages had a peculiar distribution in the respiratory passages. No haemorrhages were present in the trachea; in the main bronchi infrequent and isolated red spots were present, these became progressively more numerous in the smaller bronchi, until eventually the mucous membrane of still smaller bronchi was of a more or less uniform dark red colour owing to the presence of very numerous small haemorrhages. The smaller bronchi contained a considerable quantity of bloody froth.

Microscopic Examination: Heart.—There were haemorrhages throughout the substance of the myocard. In places there was a structureless material, probably haemoglobin, between the muscle fibres.

Liver.—Frozen sections: Stasis was present and fat in the form of a very fine, almost granular dust occurred in the liver cells. Embedded sections: The section did not stain uniformly with haemalum-eosin. Portions of the liver lobules, particularly those immediately around the central veins, had a more intense pink colour, than the peripheral portions. This is regarded as being due to haemoglobin discoloration. Blood in the larger vessels seemed to contain an excessive number of eosinophiles. No actual liver necrosis could be recognised. The cytoplasm of the liver cells was granular in appearance. Some cells contained droplets of haemoglobin and others vacuoles which varied in size. On careful

examination with a 4 mm. dry lens many of the vacuoles were seen to contain a peripheral shell showing the characteristic haemosiderin staining with Berliner Blue. The interpretation which suggests itself is that the vacuoles were actually occupied by drops of haemoglobin; towards the periphery of the drop the haemoglobin had already become changed into haemosiderin, but the greater central mass not being so changed was not stainable and disappeared during preparation of the sections, thus forming the vacuoles. Quite a number of other cells, particularly the Kupfer Stern cells, also contained fine haemosiderin granules. There was in addition a certain amount of yellowish-brown pigment, probably a non-iron-containing derivative of haemoglobin.

Kidney.—There was well-marked hyperaemia and even haemorrhages, especially in the boundary zone and in the medulla (see Fig. 3). In many cases the capillaries of the glomeruli stood out prominently. Bowman's capsule was distended with a structureless pink staining (with eosin) substance (haemoglobin) and many convoluted as well as straight tubules contained cylinders of haemoglobin (see Figs. 1 and 2). A certain amount of haemosiderosis was present and fat was seen in the form of very fine granules. These lesions could be described as those of haemoglobinuria, haemosiderosis, hyperaemia, haemorrhages and a certain amount of nephrosis.

Fig. 1.—Kidney Horse 20200 showing haemoglobin cylinders in tubules.

Spleen.—Only here and there could normal follicles be recognised, otherwise the normal splenic tissue was replaced by masses of blood.

Lung.—There was marked hyperaemia, well-marked oedema, emphysema and perivascular haemorrhages, associated particularly with the larger pulmonary vessels. There was also well-defined peribronchial haemorrhages. The bronchi themselves appeared to be in a state of contraction, the lumina being almost completely obliterated. In some cases the bronchi contained quite a number of red cells. This was due to endo-bronchial haemorrhages.

Fig. II.—As figure I, showing haemoglobin in Bowman's capsule.

Conclusion.—A fatal case of shock was produced by the intravenous injection of 300 c.c. of a haemoglobin solution prepared according to the method of Dudley and Evans from an incompatible donor.

A further supply of haemoglobin solution was obtained from another horse and 1 litre of it was injected into a compatible receiver. Apart from accelerated respirations this animal did not develop any other disturbances. In this case dialysis of the red cells was not complete (± 10 per cent. of red cells remained intact). In spite of this the dose of haemoglobin injected was undoubtedly bigger than that which proved fatal in the incompatible receiver. It is further

believed that the experiments with haemolysed blood would serve as additional and probably more efficient controls as, besides the free haemoglobin, such things as leucocytes and stromata were also injected.

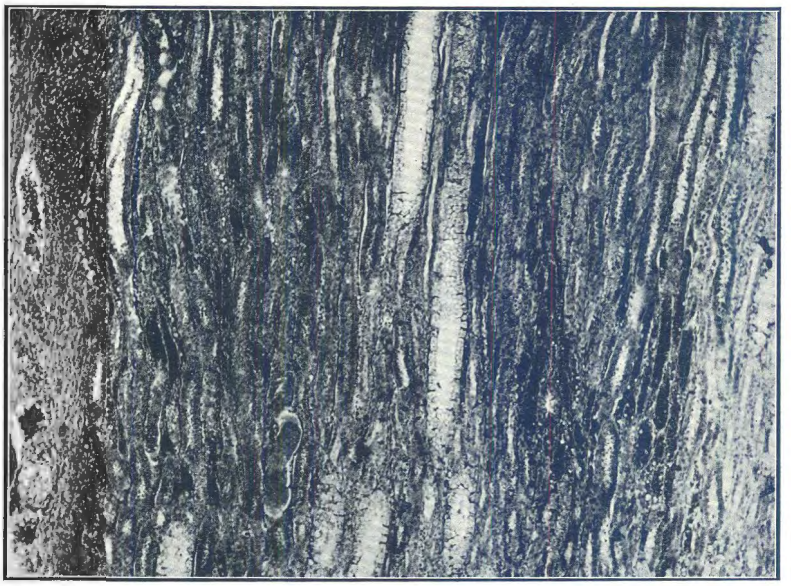


Fig. III.—As figure I, showing hyperaemia and haemoglobin cylinders in tubules of boundary zone and medulla.

GENERAL DISCUSSION.

Clinically and anatomico-pathologically this shock very strongly resembles those fatal cases of shock already described when (*a*) whole blood was transfused from an incompatible donor (20359), (*b*) when own transformed red cells were injected intravenously (20290, and (c) when haemolysed blood from an incompatible donor was injected intravenously (20258).

This resemblance is not confined to the fatal cases of shock, but clinically all the experimental cases of shock, including the non-fatal cases, are almost identical. The differences which may be present are those of degree and not qualitative. This resemblance, clinically and anatomico-pathologically, of the fatal and non-fatal cases of shock to fatal and non-fatal cases of anaphylaxis reported in the literature is most striking. This deduction is made mainly by analogy, as a search of the literature available has not revealed a complete description of anaphylaxis in equines. The main symptom present in equines is due to respiratory disturbances, especially inspiratory dyspnoea, with epistaxis. This is accompanied by sweating, increased peristalsis, which may result in frequent defaecation and even diarrhoea. The crouching movements seen in some cases, with sagging on one hindquarter is probably due to pain and is analogous to the acute pain experienced by humans suffering from transfusion shock. The experimental evidence is insufficient to make

a definite statement in connection with oliguria and anuria. The urine of the horse is normally alkaline. Therefore one would not expect oliguria and anuria to be present, to the extent it occurs in the human subject, where the acid state of the urine is held to be mainly responsible for the symptoms of uraemia. In one case where the urine was made acid by the administration of mono-ammonium phosphate and three litres of own haemolysed blood was injected intravenously, no symptoms excepting a temperature reaction, were observed for a period of three weeks after the injection. However, this aspect of the problem requires further study, when the effects of the intravenous injection of haemolysed blood from another but compatible donor and haemolysed blood from an incompatible donor, should in addition be observed in horses with an acid urine.

Pathologically the outstanding lesions present in all cases are: petechiae, ecchymoses and extravasations in mucous and serous membranes, in the epi- and endocardium, haemorrhages throughout the substance of various organs and oedema and emphysema of the lungs. To what extent the contracted state of bronchioli seen microscopically is a true indication of what was present before death is not known. Haemoglobin cylinders are present with degenerative changes in the kidneys. The liver necroses described in fatal human cases, were not seen in the horses examined. In this animal the lesions in the liver were those of marked stasis atrophy with haemorrhages, degenerative changes and a certain amount of haemosiderosis.

No systematic examination of the blood was made of cases showing shock. It is hoped to do this in the immediate future. However, in one case the counts were: red count 13 million, white count 4,900, red precipitate 60 per cent., that is after a transfusion of 10 litres of blood was made during hyperimmunisation against horsesickness. After the transfusion was completed the animal developed severe shock with haemoglobinaemia and died within 12 hours after transfusion. There were very extensive haemorrhages of the serous and mucous membranes, of the epi- and endocardium, of the various organs of the body, including the musculature of the tongue and diaphragm, of the spleen, etc. There was very marked stasis of the liver, but unfortunately no specimens were collected for microscopic examination. The differential counts were: lymphocytes 88, neutrophiles 5, eosinophiles 5, monocytes 1 and basophiles 1. This was interpreted as a case of very marked neutrophile leucopaenia, but these figures lose much in value on account of the absence of pre-transfusion counts. Nevertheless they are significant when compared with the counts from four other horses which did not show any symptoms of shock. Details of the pre- and post-transfusion counts of these four horses will be found in the accompanying table. Three out of these horses were immunised with horsesickness virus and serum on 31st October, 1933, and were hyperimmunised by transfusing 10 litres of blood from donors at the height of the horsesickness reaction on 12th January, 1934. The other horse 20680 was immunised on 7th August, 1933, hyperimmunised for the first time on 26th September, 1933, and for the second time on 12th January, 1934. The pre-transfusion counts were made on 11th January, 1934, and the post-transfusion counts on 12th January, 1934, within 12 hours after transfusion.

TABLE 5.

	Red Count.		Red Precipitate.		Total White Count.		Lympho-cytes.		Neutro-philes.		Monocytes.		Eosino-philes.		Baso-philes.	
	Pre.	Post.	Pre.	Post.	Pre.	Post.	Pre.	Post.	Pre.	Post.	Pre.	Post.	Pre.	Post.	Pre.	Post.
Horse 20775...	10·2	10·8	43	50	15·2	17·3	29	22	62	76	1	2	7	0	1	0
Horse 20778...	7·2	12·3	39	45	13·0	12·3	18	21	80	77	2	1	0	1	0	0
Horse 20779...	8·7	11·2	44	50	7·1	10·4	23	9	74	89	2	1	0	0	1	1
Horse 20680...	8·3	8·6	33	37	15·8	17·0	25	24	54	72	2	1	19	3	0	0

If one may draw any conclusions at all from these figures, then there would seem to be a tendency towards a neutrophile leucocytosis with transfusion of horsesickness blood. This may, of course, not be the case when transfusion is practised from the normal individual and before a final opinion can be given these counts will have to be repeated in properly planned transfusion experiments using compatible and incompatible donors in normal health.

In all twenty-seven horses were used in the shock experiments. Ten were used in direct whole blood transfusions; of these three were controls which received compatible blood with negative shock reactions, and seven were transfused from incompatible donors. Of these two reacted with severe shock, one of which terminated fatally, and one reacted with mild shock; two gave completely negative reactions, and in the remaining two mild disturbances were observed. The number of cases of experimentally produced shock are admittedly somewhat on the small side. It is, however, not possible to accurately control the degree of shock and for reasons of economy one is forced to work with a limited number of the large animals.

Eleven were used in experiments where haemolysed blood was injected intravenously. Of these, two received their own haemolysed blood and five received haemolysed blood from other but compatible donors. In these seven horses shock was not produced and they may be regarded as controls to the remaining four cases in which incompatible haemolysed blood was injected intravenously. Of these four, three reacted with severe shock, which was fatal in one case, and the fourth developed well-defined but mild shock. Four were used in experiments with transformed red cells. Two reacted with severe shock, with a fatal termination in one case. The other two were controls, the one receiving own red cells after standing for 24 hours, and the other, serum to which the " J "-like bacillus was added. In both of these no reactions were observed.

The 26th horse was given an intravenous injection of haemoglobin (300 c.c.), from an incompatible donor, with a fatal termination. A bigger dose of haemoglobin prepared in more or less the same way and injected intravenously into a compatible receiver, did not produce shock.

SERUM SHOCK.

In view of the negative results obtained with own haemolysed blood and compatible haemolysed blood, it is difficult to explain the cases of serum shock met with under field conditions when 400 c.c. of horsesickness hyperimmune serum is injected intravenously, on a haemolytic basis. However, on clinical grounds one would be inclined to regard this serum shock as similar to shock which occurs when incompatible blood is transfused, but until such cases become available for a complete haematological examination, it is futile to speculate on their pathogenesis.

TRANSFUSION SHOCK.

The mild disturbances which occur when incompatible blood is transfused may be due to a certain amount of intravascular agglutination which may take place, but the severe shock is due to haemolysis.

This more or less confirms the work of Lindau (1928) and others. In view of the negative results with own haemolysed blood and haemolysed blood from compatible donors, such haemolytic shock can only occur when the red cells of the donor are incompatible, and could for instance not occur with the universal donor. However, there is definite evidence (Lindau, 1928, and others) that the use of the universal donor may sometimes lead to severe disturbances and shock. An examination of this question in the light of Sabin's work on leucocytic incompatibility, already referred to, may reveal interesting results.

The results with haemolysed blood further show that incompatibility is not necessarily due to the whole erythrocyte as such, but may be due to a part of the red cell. In one case where an apparently pure solution of haemoglobin was injected intravenously into an incompatible receiver, fatal shock occurred, whilst a haemoglobin solution prepared in more or less the same way and injected in larger quantities, produced no shock in a compatible receiver. In addition, one has the evidence of the harmlessness of compatible haemolysed blood, where, besides free haemoglobin, leucocytes and stromata are injected. If it be assumed that the haematin portion of the haemoglobin molecule is non-toxic and that in accordance with the work of Borchardt and Tropp, globin is the toxic factor, this toxicity must, in view of the results already presented in this paper, be confined to incompatible bloods and globin of own blood and of compatible blood should be harmless. In that case incompatibility due to the agglutinogen factor may yet be found to be due to a difference in structure of the globin portion of the haemoglobin molecule and that such structural difference may actually be responsible for the various blood groups in different individuals of the same species. According to Poldermann (1932) there is no indication of dissimilarity of the chlorhaemins from different haemoglobins when such chlorhaemins are examined spectrophotometrically.

SUMMARY AND CONCLUSIONS.

1. According to the practical experience of veterinarians in the field shock of varying degrees of severity occurs in horses when they are injected intravenously with the usual doses (400 c.c.) of horsesickness hyperimmune serum. It is unlikely that this shock is of a haemolytic nature.

2. In one case severe shock was produced when transfusing blood from a donor having group relationships of the human universal donor. The red cells of this donor were proved to be compatible, as in addition to negative *in vitro* tests no reaction was obtained when injecting the haemolysed red cells intravenously. The serum, however, also reacted negatively in so far as shock was concerned when injected intravenously. Unfortunately this blood was not examined for leucocytic incompatibility.

3. In only a proportion of cases is severe haemolytic shock induced when 4 litres of whole blood are transfused from donors with incompatible red cells.

4. When incompatible red cells do not eventually become haemolysed disturbances of only a mild nature occur, with the transfusion of as much as 4 litres of whole blood.

5. If such incompatible red cells are haemolysed *in vitro*, then severe shock may occur when such a solution is injected in relatively small doses intravenously into an incompatible receiver (300 c.c.).

6. Own haemolysed blood injected intravenously in relatively large doses (3 litres) does not produce shock (2 cases).

7. Haemolysed blood from a compatible donor injected intravenously in relatively large doses (3 litres) does not produce shock (5 cases).

8. Red cells transformed as a result of the action of a " J "-like bacillus of Friedenreich, produce severe shock when injected intravenously into the same animal. In view of the experimental evidence that own haemolysed blood and haemolysed blood from compatible donors are not toxic, it is extremely doubtful if this shock can be satisfactorily explained on a haemolytic basis alone and from the available evidence, agglutination cannot be excluded as the aetiological factor in such cases.

9. A solution of haemoglobin, as far as possible freed from stromata, produced shock with a fatal termination in a relatively small dose (300 c.c.) when injected intravenously into an incompatible receiver. A bigger dose of haemoglobin prepared in more or less the same way did not produce shock when injected into a compatible receiver.

10. If it be assumed that the haematin portion of the haemoglobin molecule is non-toxic and that in accordance with the work of Borchardt and Tropp globin is toxic, this toxicity must, in view of the results already presented in this paper, be confined to incompatible bloods, and globin of own blood and of compatible blood should be harmless. In that case incompatibility due to the agglutinogen factor, may yet be found to be due to a difference in the structure of the globin portion of the haemoglobin molecule and that such structural difference may actually be responsible for the various blood groups in different individuals of the same species.

REFERENCES.

ANDERSON (1932). Blood Grouping in Man and Animals with Special Reference to its Occurrence in the Equine. *Veterinary Record*, No. 2293, Vol. 44, pp. 691-698.

BAKER, A. H. (1925). A Useful Preservative for Whole Blood. *The British Journal of Experimental Pathology*, Vol. 6, p. 201.

BARRET UND YORKE (1911). Ueber Hamoglobinamie. *Zeitschrift für Immunitätsforschung*, 1. Teil 12 (1912), pp. 333-352.

BAYLISS, W. M. (1920). Is Haemolysed Blood Toxic? *The British Journal of Experimental Pathology*, Vol. 1, pp. 1-8.

BORCHARDT UND TROPP (1928). Experimentelle Beiträge zur Symptomatologie der Akuten Blutzerfallskrankheiten. Swarzwasserfieber, Paroxysmale Hämoglobinurie u.s.w. *Klinische Wochenschrift*, Jr. 7, Nr. 24, 1136-1137.

BROCKMAN, H. (1911). Ueber Gruppenspezifische Strukturen des tierishen Blutes. *Zeitschrift für Immunitätsforschung*, Orig. Bd. 9, No. 1, pp. 87-116.

BURGHARDT (1933). Blutgruppen beim Pferde. *Zeitschrift für Veterinärkunde*, Jr. 45, H. 2, S. 33-52.

COCA, A. F. (1909). Die Ursache des plötslichen Todes bei intravenöser injektion artfremder Blutkörper. *Virchows Archiv.*, Bd. 196, S. 92-107.

DOAN, C. A. (1927). The Transfusion Problem. *Physiological Reviews*, Vol. 7, No. 1, pp. 1-84.

DOER, R. (1929). Allergie und Anaphylaxie. *Handbuch der Pathogenen Mikroorganismen*, Bd. 1, Teil 2.

DUDLEY, H. W., AND EVANS, C. R. (1921). A Method for the Preparation and Recrystallisation of Oxyhaemoglobin. *Biochemical Journal*, Vol. 15, No. 4, pp. 487-488.

EHRLICH UND MORGENROTH (1900). Ueber Haemolysine. *Berliner Klinische Wochenschrift*, Nr. 21, S. 453-458.

FISCHBEIN (1913). Isoagglutination in Man and Lower Animals. *The Journal of Infectious Diseases*, Vol. 12, pp. 133-139.

FORSSMAN (1925). Ein neues Krankheitsbild nach Seruminjektionen. *Biochemische Zeitschrift*, Bd. 110, S. 164-175.

FORSSMAN AND SKOOG (1925). The Reverse Anaphylaxis and the Carotal Complex of Symptoms in their Relation to China Ink. *Acta Path. et Microbiol. Scandin.*, Vol. 2, pp. 55-67.

FORSSMAN (1927). Studies of the Carotal Symptoms in Guinea Pigs after Injections of Certain Sera and Suspensions. *Acta Path. et Microbiol. Scandin.*, Vol. 3, pp. 749-771.

FRIEDENREICH, V. (1928). Investigations into the Thomsen Hemagglutination Phenomena. *Acta Path. et Microbiol. Scandin.*, Vol. 5, pp. 59-101.

FRIEDENREICH, V. (1930). The Thomsen Hemagglutination Phenomenon. *Contributions for the University Institute for General Pathology, Copenhagen*, pp. 9-138.

FRIEDENREICH, V. (1930). The Thomsen Hemagglutination Phenomenon (Intravital Effects of Transformed Corpuscles in Guinea Pigs). *Contributions from the University Institute for General Pathology, Copenhagen*, pp. 109-118.

HERLYN, K. E. (1928). Ueber Blutgruppen bei Tieren. *Zuchtungskunde*, Bd. 3, Heft. 8, S. 377-397.

HESCH, M. (1932). Die Entwicklung der Blutgruppenforschung, 1901-1931. *Handbuch der Blutgruppenkunde*, S. 1-16.

HIRSZFELD UND PRZESMYCKI (1921). Untersuchungen über die normale Agglutination, Mitt. IV. Ueber die Isoagglutination bei Pferden. *Przeglad epidemjol*, Bd. 1, H. 6, S. 577. Autoreferaat (1922). *Berichte über die Gesamte Physiologie*, B. 12, S. 304.

INGEBRIGSTEN, R. (1912). Die Bedeutung der Isoagglutinine für die Schicksal homoplatstisch transplantierter Arterien. *Münchener Medic. Wochenschrift*, S. 1475-1476, und *Jurnal of Expt. Medicine*, Vol. 16, pp. 169-177.

KALMER AND MOTOMATSU MATSUMOTO (1920). Natural Antihuman Hemolysins and Hemagglutinins in Horse Sera in relation to Serum Therapy. *Journal Immunology*, Vol. 5, pp. 75-88.

KLEIN, A. (1902). Beitrag zur Kenntnis der Agglutination roter Blutkörperchen. *Wien. Klin. Wocht.*, S. 413-418.

KRUMBHAAR, E. B. (1928). The Erythrocyte in *Special Cytology*, Vol. 1, edited by Cowdry. Hoeber, New York, p. 289.

LAGUNA, S. (1930). Ueber scheinbares Fehlen des A-Receptors in der Gruppe AB. *Klinische Wochenschrift*, Jr. 9, Nr. 12, p. 547.

LANDSTEINER, K., AND VAN DER SCHEER, J. (1924). Serological Examination of a Species Hybrid, 1. On the Inheritance of Species-specific qualities. *J. of Immunology*, Vol. 9, pp. 221-226.

LEMKE, R. (1925). Pathologisch Anatomische Befunde bei Todesfällen nach Bluttransfusionen. *Virchows Archiv. für Path. Anatom und Physiolog. und für Klinische Medizin.*, B. 257, S. 415-429.

LINDAU (1928). Reaktionen nach Bluttransfusion. *Acta Path. et Microbiol. Scandin.*, Vol. 5, pp. 382-422.

MASON, J. H. (1934). Thomsen's Hemagglutination Phenomenon. Isolation of a "J"-like Bacillus. *Onderstepoort Journal of Veterinary Science and Animal Industry*, Vol. II, No. 1, pp. 25-27.

NEWODOFF, A. P. (1927). Die Blutgruppen an Pferden. *Mikrobiologits. Journal*, 5, H.2 (Russisch). Ref. *Zbl. Bakter.*, 1. Ref. Bd. 90, 1928, p. 157.

OTTENBERG, R. (1911). Transfusion and the Question of Intravascular Agglutination. *Journal of Experimental Medicine*, Vol. 13, pp. 425-438.

OTTENBERG, R., AND FRIEDMAN, S. S. (1911). Studies in Isoagglutination, II. The Occurrence of Grouped Isoagglutination in the Lower Animals. *Journal of Experimental Medicine*, Vol. 13, pp. 531-535.

OTTENBERG, R., AND KALISKI, D. J. (1913). Accidents in Transfusion. *Journal of the American Medical Association*, Vol. 61, No. 24, pp. 2138-2140.

POLDERMANN, J. (1932). Ueber die Einheitlichkeit der Prosthetischen Gruppe der Hämoglobine verschiedener Herkunft. *Biochemische Zeitschrift*, Vol. 251, pp. 452.

SCHERMER (1928). Untersuchungen über die Blutgruppen des Pferdes. *Zeitschrift für Immunitätsforschung*, Bd. 58, pp. 130-142.

SCHERMER, A., UND HOFFERBER, O. (1928). Individualitäts-reaktionen (Isohämagglutination Isolysis, Heteragglutination und Heterolysis) des normalen Pferdeblutes. *Archiv. Wiss. Prakt. Tierheilkunde*, Bd. 57, pp. 77-98.

SCHIFF, F. (1929). Die Technik der Blutgruppenuntersuchung für Kliniker und Gerichtärzte. Zweite Auflage. Berlin. Julius Springer.

SCHWARZ, M. (1926). Beiträge zur Kenntnis der Isoagglutinine in Pferdeblut. *Zeitschrift für Immunitätsforschung*, B. 48, S. 79-95.

SCOTT, W. M. (1931). Anaphylaxis and Related Phenomena. *A System of Bacteriology (Medical Research Council)*, Vol. 6.

STEFFAN PAUL (1932). Handbuch der Blutgruppenkunde.

THEILER, A. (1918). Acute Liver Atrophy and Parenchymatous Hepatitis in Horses. *5th and 6th Reports of the Director of Veterinary Research*, pp. 9-164.

THOMOFF, Z. (1930). Die Blutgruppen des Pferdes. *Archiv. Wiss. Prakt. Tierheilkunde*, Bd. 61, pp. 433-444.

THOMSEN, O. (1927). Ein Vermehrungsfähiges Agens als Veränderer des isoagglutinatorischen Verhaltnis der roten Blutkörperchen einer bisher unbekannte Quelle der Fehlbestimmung. *Zeitschrift für Immunitätsforschung*, Bd. 52, S. 85-107.

THOMSEN, O. (1929). Zwei Fälle von Mutmasslich zur AB-gruppe gehörigen Erwachsenen mit nicht nachweisbarem A. Receptor. *Klin. Wochenschrift*, Jr. 8, Nr. 23, S. 1075.

THOMSEN, O. (1932). Die Serologie der Blutgruppen. *Handbuch der Blutgruppenkunde*, S. 100.

VAN HERWERDEN, M. H., AND BOELE-NYLAND, T. Y. (1930). Investigation of Blood Groups in Holland. *Reprint from Proceedings*, Vol. 33, No. 6, 1930.

VON DUNGERN, E., UND HIRSCHFELD., L. (1909). Ueber Nachweis und Vererbung biochemischer Strukturen. *Zeitschrift für Immunitätsforschung*, Orig. Bd. 4, S. 531-546.

VON DUNGERN UND HIRSCHFELD (1910). Ueber gruppenspezifische Strukturen des Blutes. *Zeitschrift für Immunitätsforschung*, Orig. Bd. 8, Nr. 1, S. 526-562.

WILDEGANS, H. (1930). Die Todesfälle nach Bluttransfusionen. *Deutsche Medizinische Wochenschrift*, Jr. 56, No. 48.

WITTS, L. J. (1929). A Note on Blood Transfusion with an Account of a Fatal Reaction. *Lancet*, Vol. 216, pp. 1297-1299.