HAEMOLYTIC DISEASE OF NEWBORN FOALS DUE TO ISO-IMMUNIZATION OF PREGNANCY

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(With Plate 14 and 2 Figures in the Text)

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I. INTRODUCTION

The clinical condition of icterus gravis neonatorum has long been recognized in both human and veterinary medicine. The human condition is included as one of the manifestations of haemolytic disease of the newborn which is now known to be due to iso-immunization of the mother by one or more of the foetal red-cell antigens of the *Rh* group. The elucidation of the aetiology of the condition in the human infant suggests the possibility of a comparable pathogenesis for the disease in other animal species.

To our knowledge the first work reported on the subject of iso-immunization of pregnancy in animals was that of Caroli & Bessis (1947*a*), who drew attention to the condition of icterus gravis in newborn mules and put forward their hypothesis that it was due to an iso-immunization of the mare dam by the foetal red-cell antigens inherited from the donkey sire. The full investigations carried out during the foaling season 1947 were reported by Caroli & Bessis (1947*b*, *c*) and Bessis & Caroli (1947). In a comprehensive paper they described the serology, haematology, pathology and possible lines of treatment. A comparative study was also made of the disease as it occurs in human infants, newborn mules and that produced experimentally in the young rat by ingestion of rabbit anti-rat red-cell sera. Further observations on the 1948 foaling season are also to be published in the near future by these authors.

One of us (R. R. A. C.) had the honour of being invited to France by Doctors Bessis and Caroli to see the disease as it occurs in newborn mules. The opportunity was also taken to investigate the possibility of applying the various serological techniques used in human *Rhesus* factor investigations to the diagnosis of the disease in newborn mules.

In this paper we describe observations which show that haemolytic disease in newborn thoroughbred foals may be due to intraspecies blood-group differences which are capable of giving rise to iso-immunization of pregnancy.

Eight cases of jaundice in newborn foals have been examined. The red blood cells of the foals in six of these cases were shown to be sensitized *in vivo* by maternal immune iso-antibodies which were also demonstrated in the serum of the dam in each case. Thus, in these cases, there was every indication that the mares had been immunized by foetal red-cell antigens inherited in each case from the respective sire. The jaundice in the other two foals was shown not to be due to iso-immunization of pregnancy. The cases have been studied from the point of view of their clinical manifestations, serology, haematology and pathology. A possible programme for specific treatment is discussed at the end of the paper.

We realize that our investigations are far from complete; yet we feel that the observations could form a starting-point for a much fuller and better controlled investigation which would not only be of

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https://doi.org/10.1017/S0022172400036573 Published online by Cambridge University Press

interest to the veterinary profession but also, as a study in comparative pathology, would be of interest to workers engaged in the investigation of the human disease. Such a study might well help in the elucidation of the many problems still unsolved in the pathogenesis of haemolytic disease in newborn infants.

This preliminary investigation has been carried out under difficulties. Up to the present we have had no experience in blood-grouping horses nor did we possess any grouping sera for these animals. Difficulties have been encountered in obtaining blood samples from normal thoroughbred horses and at the time of publication we have not even been able to obtain a blood sample from the respective stallion of each foal, an examination of which is really necessary to complete the investigation.

We ourselves are also very ignorant of the normal blood picture and histological appearance of the tissues of normal foals of comparable ages to those described in this paper, and we have been unsuccessful in procuring sufficient control material.

iso-immunization of pregnancy was shown to be responsible for the pathogenesis, suggest that this process may be responsible for some cases of non-infective haemolytic disease in newborn foals.

These non-infective cases exhibit certain clinical features which serve to establish a definite clinical syndrome. The foals are normal at birth; thereafter, at periods varying from a few hours to the fifth day after birth, they show progressive lethargy and weakness, icterus and pallor of mucous membranes, with, in acute cases, haemoglobinuria. Those in which signs appear within the first 3 days of life are usually affected more severely and die in 24–36 hr. from the onset of lethargy. Older foals are often affected less severely and some may recover with good nursing only.

The horses studied in this paper were thoroughbreds. In all cases parturition was uneventful, and, except in case 1 which was 14 days overdue, at term. The dams were in normal health.

In the six cases in which the disease process can be

Table 1. *Summary of the principal clinical findings*

Case no.	Aetiology	Clinical classification	Age when clinical condition first observed	Haemoglobinuria	Pallor	Icterus	Cardiovascular signs	Outcome
1	Iso-immunization of pregnancy	Peracute	1½ days	+++	+	+++	+++	Died on 13th day after repeated transfusions of dam's washed erythrocytes
2	Iso-immunization of pregnancy	Peracute	15 hr.	+++	++	++	—	Died when 20 hr. old before treatment commenced
3	Iso-immunization of pregnancy	Peracute	8 hr.	+++	+++	+	—	Found dead
4	Iso-immunization of pregnancy	Acute	4 or 5 days	+	+	+++	+++	Died when 5½ days old after two transfusions of whole blood, shown later to be incompatible
5	Iso-immunization of pregnancy	Subacute	5 days	—	+	++	++	Recovered without transfusion
6	Iso-immunization of pregnancy	Subacute	4 days	—	+	++	++	Recovered without transfusion
7	Probably bacterial septicaemia	—	Birth	—	—	+++	—	Died when 24 hr. old
8	Coliform septicaemia	—	18 hr.	—	—	++	—	Died when 36 hr. old

II. GENERAL OBSERVATIONS AND METHODS OF INVESTIGATION

(*a*) *Clinical observations*

Syndromes in newborn foals characterized by jaundice, lethargy and extreme weakness with early death have been recognized for a number of years. Some are bacterial in origin (cases 7 and 8 in this report probably fall into this category), but there remain cases from which no bacterial organism can be recovered. The six cases reported here, in which attributed to iso-immunization of pregnancy, the signs have been those of acute and severe anaemia due to intravascular haemolysis. Pallor of the mucous membranes was marked and before death extreme. There was progressive lassitude and weakness of the musculature. The foals failed to suck normally, although they would suck if supported under the dam. Finally, the foals became too weak to move. The body temperatures were normal. In the severe cases haemoglobinuria has been the first sign noted, but in the milder cases no haemoglobinuria

https://doi.org/10.1017/S0022172400036573 Published online by Cambridge University Press

has been observed. The respiration has been normal except when terminal cardiovascular insufficiency developed and then hyperpnoeic breathing with frequencies up to 80 per min. have been observed.

The main clinical signs have been those referable to the cardiovascular system; they have closely followed the haematological findings. Tachycardia has been present constantly, and in severe cases the heart frequency has been raised from the normal level of about 60 to frequencies up to 120–180 beats/min. There was marked cardiac palpitation, if this term may be used in the objective sense. On auscultation the heart sounds were markedly augmented both in amplitude and in the area over which they were audible. In the severe cases there was a marked positive cardiac jugular pulse and in such cases the peripheral arterial pulse was imperceptible.

On the basis of the clinical signs the cases observed can be conveniently divided into peracute, acute and subacute types. The details of these cases are summarized in Table 1.

The two other foals showing marked jaundice, but in which no evidence of iso-immunization could be found, probably died of a bacterial septicaemia. They served as good control cases and their clinical findings are also included in Table 1. Both these cases showed lassitude and icterus within 24 hr. of birth but no evidence of acute anaemia.

Specific transfusion therapy was adopted in only two of the cases reported and both these foals died. Due mainly to our lack of knowledge of their blood groups, transfusion in the horse is purely an experimental measure at the present time.

The expedient adopted in case 1 was that of transfusing the foal with the dam's washed erythrocytes, as presumably the maternal antibody in the foal's serum would not react with the mare's cells. However, in this case, which was the most severe treated, the beneficial response to the repeated transfusions was not maintained. Another foal (case 4) was transfused with the whole citrated blood of a donor horse, but this was subsequently found to be incompatible with the dam's serum, probably due to the immune iso-antibody.

The full clinical history of each case, whose pathology is reported in this communication, is being published in a separate paper.

(*b*) *Serology*

The serological procedures employed in investigating haemolytic disease in newborn infants have been applied in this study with the necessary modifications.

Assuming that the pathogenesis of the disease in the two species depended on similar processes, we expected to find antibodies of a fairly high titre in the mare's serum which would react with the red blood cells of the sire and with the cells of other horses carrying the same particular antigen or antigens concerned. Such a finding would indicate that the dam had been immunized by antigens which the foetus possessed by inheritance from the sire.

On the same assumption the actual haemolytic crisis in the foal would be due to an *in vivo* sensitization of the foal's red cells by the maternal antibody.

The serological investigations carried out have been directed towards these two aspects of the disease process.

(1) *Examination of the serum of the dams for immune antibodies which react with the red cells of the sire or other horses which possess the antigen or antigens in question*

Unfortunately, up to the present, we have not been able to obtain blood samples of the various sires. However, it is hoped that these will be obtained in due course so that we may test them against the dams' sera. Also, the fact that we were unable to classify horses according to their main blood groups, which are known to exist, made it impossible for us to select red cells to test the dams' sera, which we could be sure were compatible in all respects, except for the particular antigens concerned in the iso-immunization.

On account of these facts, the best we could do was to examine the sera of the mares who had jaundiced foals and also the sera of four control normal horses, against a panel of horse cells chosen at random but including the cells of certain of the mares which had jaundiced foals.

The sera were examined for their agglutinin content using serial dilutions in 0·1 c.c. amounts and an equal volume of a 2% suspension of washed horse cells. After incubation at 37° C. for 1 hr. the deposited cells were read for agglutination by removal with a pipette and examination on a slide under the low-power microscope.

The sera were also examined for non-agglutinating 'incomplete' or sensitizing antibodies. These tests were performed using a rabbit anti-horse-globulin serum specially prepared for this test after the method of Coombs, Mourant & Race (1945). Diamond's albumin tube test (Diamond & Denton, 1945) was also used for demonstrating these non-agglutinating sensitizing antibodies. This reaction tended to give results similar to those obtained by the indirect antiglobulin sensitization test, but in our hands the reading of the results was difficult on account of pseudo-agglutination.

The results obtained by the direct agglutination and indirect antiglobulin-sensitization tests are shown in Table 2. In this table the recorded results of the interactions of the various sera to a titre higher than 8 with a given red-cell suspension have been printed in darker type as probably indicating the

Table 2. *The reactions of anti-red cell iso-antibodies present in the sera of mares giving birth to foals with haemolytic disease*

Serum of	Red cells of: Thoroughbred mare case 4	Thoroughbred mare case 5	Thoroughbred mare (P)	Thoroughbred mare AJ 6	Grey thoroughbred mare	Thoroughbred mare case 3	Cob mare	Thoroughbred mare case 6	Thoroughbred mare case 2	Thoroughbred gelding NM	Thoroughbred mare IAP	Thoroughbred gelding D	Shetland Shire cross S	Shetland Shire cross P
(*a*) Interaction by ordinary agglutination test in a saline medium														
Case 1	0	0	8	0	8	0	**128**	**16**	4	4	**128**	**16**	8	**64**
Case 2	**1024**	**512**	**512**	**1024**	**16**	4	**32**	**256**	0	2	**64**	4	**1024**	**1024**
Case 3	0	2	0	0	2	0	4	0	4	4	**16**	**16**	4	**16**
Case 4	0	0	0	2	2	2	0	0	0	4	8	0	8	8
Case 5	2	0	2	0	2	4	4	4	8	8	**16**	**64**	8	**64**
Case 6	0	4	8	2	2	4	**16**	0	0	8	4	8	8	8
Case 7 control	0	0	0	0	2	0		0		0	2	0		0
Cob mare control	0	0	0	0	0	2	0	0	2	0	2	0	0	0
Thoroughbred mare IAP control	0	0	0	0	0	0	0	2	0	0	0	0	0	0
Shetland pony (stallion) control	2	2	2	0	0	2	2	0	4	2	2	4	0	0
New Forest pony mare control	0	0	0	0	2	0	0	0	0	0	0	0	0	4
(*b*) Interaction by indirect antiglobulin sensitization test														
Case 1	0	0	8	0	< 8	0	**512**	**128**	**> 32**	**> 64**	**2048**	**> 256**	**512**	**2048**
Case 2	**1024**	**2048**	**2048**	**512**	**> 256**	**> 32**	**1024**	**1024**	0	**16**	**2048**	**> 64**	**1024**	**2048**
Case 3	0	2	0	0	4	0	**32**	**32**	**> 32**	**16**	**128**	**128**	**32**	**64**
Case 4	0	0	0	0	< 4	**> 32**	0	0	**> 32**	**> 64**	**64**	**> 64**	**32**	**64**
Case 5	0	0	0	0	0	0	< 4	**16**	**> 128**	**> 64**	**256**	**1024**	**256**	**512**
Case 6	0	8	4	**16**	**16**	**32**	< 16	0	**> 32**	**32**	**64**	**64**	**32**	**16**
Case 7 control	0	0	0	0		0		0		0		0		0
Cob mare control	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thoroughbred mare IAP control	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Shetland pony (stallion) control	0	0	0	0	0	0	0	2	4	0	2	0	0	0
New Forest pony mare control	0	0	0	0	0	0	0	0	0	0	0	0	0	0

The figures given in this table represent the titres of the interactions. The sign > means a titre greater than... and < a titre less than....

The interactions, which we consider could be taken in the absence of other relative data, to indicate the presence of immune iso-antibodies, have been printed in darker type.

presence of immune iso-antibodies. Titres of 8 and below may well represent interactions due to normal serum iso-antibodies. This titre of 8 was chosen after examination of these few early results, but it is quite possible that some normal serum iso-antibodies may be present to a titre higher than this.

It can be seen that the sera from five control horses (including control case 7, the dam of a foal dying with jaundice presumably of infective origin), contained no immune iso-antibodies, while the sera of the other six dams all contained what are probably immune iso-antibodies to certain red cells of the panel tested. The distribution of the positive reactions indicate that more than one antigen-antibody system is concerned. As yet the specificities of these immune antibodies have not been worked out; this awaits further investigation.

It is, of course, significant that the red cells of many of these dams are themselves negative when tested against these antisera. The marked enhancement of these reactions by the indirect antiglobulin-sensitization technique is also a very strong indication of their immune nature.

(2) *Serological examination of the cells of the foals for* in vivo *iso-sensitization*

The following methods were investigated for demonstrating *in vivo* iso-sensitization of the red cells of foals with maternal antibody:

(i) Direct antiglobulin-sensitization test.
(ii) Diamond's albumin tube test.
(iii) Examination for direct 'auto-agglutination'.

(i) *The direct antiglobulin-sensitization test.* This test, as used for the diagnosis of haemolytic disease in human infants (Coombs, Mourant & Race, 1946), may be applied very satisfactorily to the diagnosis of this condition in foals.

The principle of the test is the use of an anti-horse-globulin serum which will agglutinate horse red blood cells only when they have been sensitized by an iso-antibody, and thus have horse antibody globulin adsorbed at some points of their surface.

Preparation of anti-horse-globulin serum for this test. Rabbits are injected with horse serum so as to produce a rabbit anti-horse-serum precipitating serum. Once a good precipitating serum is obtained, it is necessary, after inactivation, to absorb it with a panel of normal horse red cells which have been washed at least six times in saline to remove all the horse plasma proteins. When the serum is absorbed free of agglutinins for normal horse cells it is ready for use in this test and may be preserved by the usual methods for preserving antisera.

Method of performing direct antiglobulin-sensitization test. A drop of the foal's blood is added to a small tube containing 0·6 % sodium citrate in physiological saline. The cells are centrifuged down and washed three times in saline to remove the plasma. To a drop of a 2–4 % suspension of the washed cells is added a drop of a suitable dilution of the rabbit anti-horse-globulin serum. This may be done on a tile at room temperature or in a small tube which is then incubated at 37° C. If the cells have been sensitized *in vivo* by maternal antibody they agglutinate on the addition of the rabbit antiglobulin serum.

By this method the cells of the foals of cases 1–6 were shown to be sensitized (see Table 3). The cells of cases 7 and 8 were not sensitized, and in both these cases the clinical observations, haematology and absence of maternal antibody made iso-immunization unlikely as the cause of the jaundice (see Tables 1–3).

We have not had any other normal thoroughbred foals' cells to test, but the red cells of more than 20 adult horses have all been negative to this reaction. The cells of more than 12 normal mule foals were negative to this reaction. See p. 408.

(ii) *Diamond's albumin tube test.* A small drop of the foal's oxalated blood was placed in a small tube and after the cells had sedimented at 37° C. the supernatant plasma was removed and replaced by two drops of 30 % bovine albumin solution. The tubes were shaken up and after resedimenting at 37° C. read for agglutination.

Because horse cells tend to pseudo-agglutinate in their own plasma, the above procedure was repeated, but in this case the bovine albumin was added only after the cells had been washed twice with saline to remove the plasma.

The results obtained, using Diamond's method of examining the washed and unwashed cells, are recorded in Table 3. Unwashed sensitized cells were agglutinated when suspended in the albumin medium. This medium also tended to inhibit the non-specific pseudo-agglutination of normal horse cells in their own plasma or serum. However, even in this medium the deposited unwashed cells did not give a clear homogeneous negative picture on examination, and although this appearance was different from true agglutination, we did not find the reading of the tests easy. It is probable that the results would have been clearer, although perhaps weaker, if pains had been taken to remove as much of the residual plasma as possible before adding the albumin, and if a concentration of bovine albumin other than 30 % had been used. In the Diamond test the final concentration of albumin in the tubes would have been about 20 %.

When the albumin medium was added to the deposits of washed cells, the sensitization of the cells of cases 1, 3, 5 and 6 by maternal antibody was not demonstrated by agglutination.

(iii) *Examination for direct 'auto-agglutination'.* The red cells of horses cannot be examined for specific 'auto-agglutination' (strictly an iso-agglu-

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Table 3. *Methods investigated to detect* in vivo *iso-sensitization of the red cells of foals with haemolytic disease due to iso-immunization of pregnancy*

Methods used to detect *in vivo* iso-sensitization	Red cells from: Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Control Case 7	Control Case 8	Control normal Horse C	Control normal Horse G	Control normal Horse T
Direct antiglobulin-sensitization test	++	++	++	++	++	++	—	—	—	—	—
Diamond's albumin tube test (albumin added to unwashed sedimented cells after the supernatant plasma was removed)	++ P	++	—	++	+	+	—	—	? P	? P	? P
Diamond's albumin tube test (albumin added to twice washed sedimented cells after the supernatant saline was removed)	? P	++	—	+	—	—	—	—	—	—	—
Examination for true 'auto-agglutination' after washing cells twice in saline	—	++	—	(+)	—	—	—	—	—	—	—
Examination for 'auto-agglutination' or clumping of unwashed cells in their own plasma	++ P	++	++	++	++ P	++ P	— P	++ P	++ P	++ P	++ P

The tests were performed at room temperature and at 42° C.

++ = very strong agglutination; + = definite agglutination;
(+) = very weak agglutination; ? = uncertain whether agglutination present;
P = whole picture complicated by typical appearance of pseudo-agglutination.

tination) while still in the presence of their undiluted plasma or serum, as even with the cells of normal horses a non-specific 'auto-pseudo-agglutination' or clumping of the cells may be evident. This non-specific clumping of the cells in their own plasma also occurs at 42° C.

For this reason horse cells must be washed free of their own plasma before specific 'auto-agglutination' can be demonstrated. The cells of two out of the six cases reported, which were shown to be sensitized by the direct antiglobulin-sensitization test, showed true 'auto-agglutination' after the cells had been washed twice in saline. Thus, when true 'auto-agglutination' does occur it may be taken as diagnostic of this disease, but there may be many cases in which the red cells are sensitized but fail to show 'auto-agglutination' in a saline medium after washing (see Table 3).

During the visit of one of us (R. R. A. C.) to France, these same serological procedures were investigated in collaboration with Mlle Gorius for their application in the diagnosis of the disease in mules. The findings reported here were amply confirmed. Five cases of the disease were examined as well as over twelve normal mule foals; in the latter the serological results were completely negative. In the case of Diamond's albumin test in which a 30 % solution of bovine albumin was added to the sedimented cells in their own plasma, the results were better than those obtained with the horse foals and gave similar results to those obtained with the direct antiglobulin-sensitization test.

(c) *Haematology*

All the peripheral-blood examinations were performed on venous blood using a mixture of ammonium and potassium oxalate as anti-coagulant. The blood specimens had to be transported several miles from the field to the laboratory and on several occasions as much as 24 hr. elapsed before the specimens reached the laboratory. Erythrocyte, leucocyte and reticulocyte counts were performed only on specimens which had been in contact with the oxalate mixture for less than 5 hr.

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The haemoglobin was estimated by the oxyhaemoglobin photocolorimetric method, using 0·1 c.c. British Standards pipettes for dilution. For the reticulocyte counts, preparations were made by placing a small drop of blood on a glass slide on which a drop of 0·5 % alcoholic brilliant cresyl blue had been allowed to dry. The drop of blood and dry stain were thoroughly mixed and smears were made after the cells had been in contact with the stain in a moist Petri dish for 5 min.

The observations are recorded under the individual cases, while the results of the series of examinations on cases 1, 5 and 6 are shown in Figs. 1 and 2.

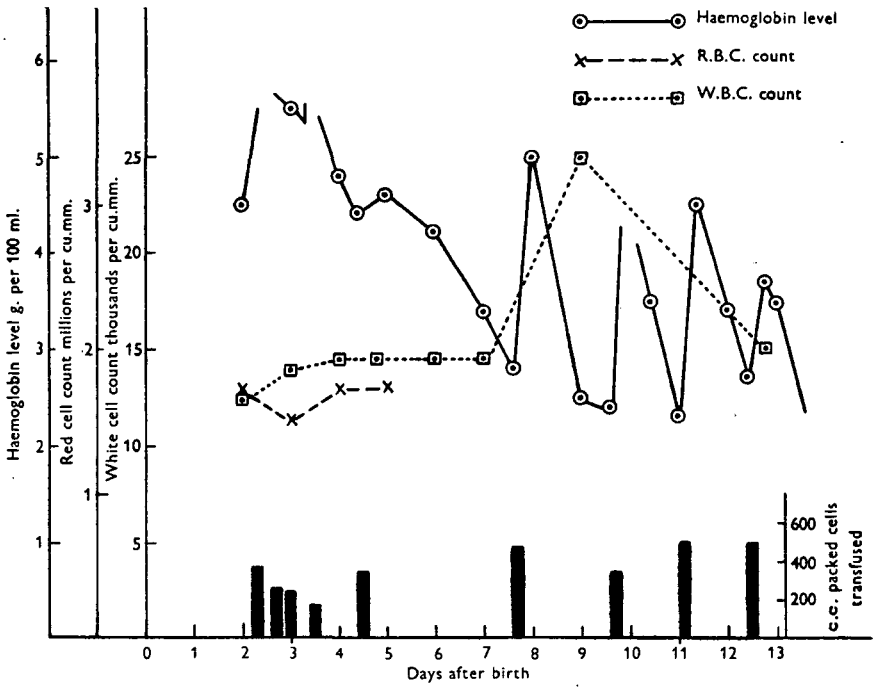

Fig. 1. Haematological response of case 1.

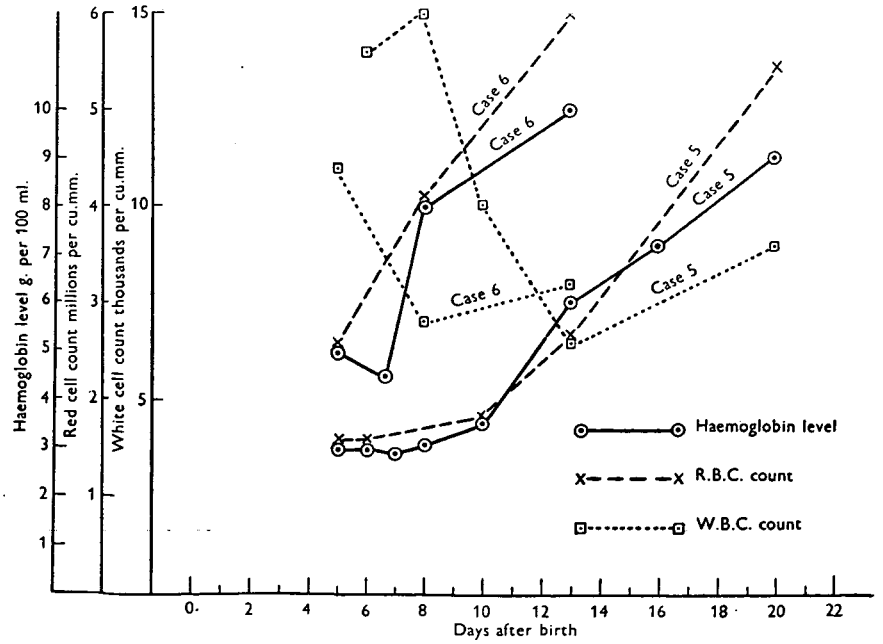

Fig. 2. Haematological response of cases 5 and 6.

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(*d*) *Morbid anatomy*

Tissues for histological examination were placed in 10 % formol saline at the time of autopsy. They were fixed a further 12 hr. in formol sublimate, dehydrated in alcohol, cleared in chloroform and embedded in paraffin. After treatment with picric alcohol the sections were stained with Ehrlich's haematoxylin and eosin and, when necessary, by McFarlane's modification of the picro-Mallory stain (McFarlane, 1944), the Prussian-blue stain for iron pigment, Mann's eosin-methylene blue stain, and by Lendrum's phloxine-tartarazine methods (Lendrum, 1947). The bone-marrow tissue which was obtained from the middle of the femur, except in case 3, where it was obtained from the middle of the tibia, was fixed in formol-Zenker and stained by Barrett's bone-marrow stain (Barrett, 1944). Frozen sections were stained for lipoid with Scharlach R and Nile blue sulphate.

III. INDIVIDUAL CASES

Case 1

Clinical observations

Haemoglobinuria was the first sign noted when the foal was 24 hr. old. Twelve hours later the mucous membranes were definitely icteric, but pallor was not marked at this time. The foal was rather lethargic although still sucking. Eight hours later it was prostrate.

Transfusions of dam's washed erythrocytes were given on nine occasions; the volume of packed cells given at each transfusion is shown in Fig. 1, the cells being given usually as a 40–50 % suspension in saline. There was a remarkable and dramatic improvement after each transfusion, but the effects lasted only about 24–48 hr.; the period getting shorter after each successive transfusion. Presumably the cells were not being maintained. A tenth transfusion was then given of whole blood from a donor horse whose cells were not capable of being sensitized by the maternal antibody or the high-titred antibody in the foal's plasma. However, the beneficial effects resulting from this transfusion also were not maintained and the foal finally died on the thirteenth day of the disease. No transfusion reactions were noted during or after any of the transfusions.

The full clinical data of this and the subsequent cases are being reported in another paper.

Serology

The foal's red cells after washing in saline showed no 'auto-agglutination' but were shown to be sensitized by the direct antiglobulin-sensitization test. A sample of blood taken 36 hr. after the fifth transfusion showed no sensitization of the cells, the majority of the cells presumably being the transfused cells of the mother. When the foal died on the thirteenth day, its cells were strongly sensitized with antibody. From this observation it would seem that although the dam's cells were not sensitized by the maternal antibody in the foal's plasma, they were, nevertheless, of a different group from those of the foal, which became immunized by the repeated transfusions, with the result that eventually the transfused dam's cells also became sensitized. This may possibly explain why the beneficial effects of the transfusions were not maintained.

Immune sensitizing antibodies with the specificity shown in Table 2 were present in the dam's serum and milk and foal's plasma. The antibodies free in the foal's own plasma reacted with the cells of the thoroughbred (I.A.P.) mare to a titre over 256.

Haematology

The results of the haematological examinations performed on this case are shown in Fig. 1.

Free haemoglobin was present in the plasma of all specimens brought to the laboratory. This was not an artefact due to prolonged contact with the anticoagulant as it was observed within a few minutes of venipuncture once the cells had begun to settle. The graph shows with what difficulty the haemoglobin was maintained even at the low level of 3–4 g./100 ml. Despite the haemoglobin remaining at this low level for almost 2 weeks, the highest reticulocyte count recorded was 0·3 %, and on most days no reticulocytes could be found. Normoblasts were never found in the peripheral circulation.

The erythrocytes were always small, of uniform size, showing no polychromatophilia and no poikilocytosis. The majority of these cells were conceivably transfused cells.

The leucocyte count was usually between 12 and 15 thousand per cu.mm., the cells being predominantly neutrophil polymorphs with numerous non-segmented cells.

Unfortunately, smears of bone-marrow taken at post-mortem showed too much autolysis to permit an accurate differential count. Sufficient cytological detail remained, however, to show that the marrow was predominantly erythropoietic, there being approximately three times as much erythropoietic as leucopoietic tissue. The vast majority of the erythropoietic cells appeared to be normoblasts and late erythroblasts, but earlier forms perhaps showed too much autolysis to afford recognition. Besides a small quantity of fat, abundant greyish green granules of pigment were scattered free in the matrix of the smears or in occasional reticular macrophages. Most of these granules showed the presence of iron by the Prussian blue reaction, but some remained a golden yellow colour and may have represented bile pigment.

Morbid anatomy

Macroscopic findings

The autopsy was carried out within 4 hr. of death. The animal weighed 65 kg. There was generalized icterus. The lungs showed small haemorrhagic areas 0·5 cm. in diameter, but were fully crepitant. The heart was pale and enlarged, weighing 1·1 kg. The myocardium showed greyish green infarcts in the papillary muscle of left ventricle and right atrium 0·8 cm. in diameter. The liver appeared normal and weighed 2·9 kg. The spleen was small, contracted and weighed only 250 g. The kidneys were about three times the normal weight (420 g. each), and soft, with a marked gun-metal colour staining of the cortex. The adrenals appeared normal. The cerebral cortex was slightly icteric but this was less marked in the white matter. The cerebral spinal fluid and meninges were icteric.

Microscopic findings

Bone marrow (mid-femur). The haemopoietic tissue is increased in amount and is largely erythropoietic. The proportion of the more immature cells of the erythrocyte series is high; primary erythroblasts and early and late normoblasts are abundant. Myelocytes in all stages of development are plentiful. Slight haemosiderosis and some haemorrhages are present (Plate 14, Fig. 1).

Liver. The portal spaces are considerably enlarged, owing to the presence of swollen cells with pale greyish brown vacuolated cytoplasm, proliferating 'bile pseudo-canaliculi' and young fibroblasts. An associated loss of liver cells marks the condition as a true 'cirrhosis'. The swollen cells are most numerous at the boundaries of the lobules between disintegrating liver-cell columns, where they often completely fill the enlarged, distorted sinusoids. In the Prussian blue test many, but not all, of the swollen cells stain an indistinct greenish blue colour, and a few contain definite blue granules. They are almost certainly swollen macrophages containing altered blood pigment. Isolated dead or dying liver cells or more rarely small groups of them, with swollen granular eosinophilic cytoplasm and nuclei in various stages of lysis, are scattered throughout the lobules but are most numerous at the periphery where all the liver cells appear degenerate. A few of these minute necroses are infiltrated with polymorphonuclear leucocytes. There is considerable haemosiderosis limited to the Küpffer cells, some of which also contain a few erythrocytes. Fine bile thrombi are present in the majority of the bile canaliculi, but not in the bile ducts which appear normal (Plate 14, Figs. 3 and 4).

Spleen. The pulp shows slight to moderate engorgement, with a marked excess of polymorphonuclear leucocytes. The Malpighian bodies are small and poorly defined, and many are partially or completely replaced by almost acellular, somewhat hyaline material showing a faint fibrillar mesh in the picro-Mallory preparation. The fibrillae are most conspicuous at the periphery where they merge with the reticulin of the pulp. A few lymphocytes, erythrocytes and spindle-shaped nuclei, often compressed and distorted, are embedded in the hyaline material. The few comparatively normal Malpighian bodies contain many nuclear fragments. There is severe haemosiderosis, the iron being diffuse or in granules, usually intracellular but sometimes free in the pulp. A few macrophages contain ingested erythrocytes.

Lymph nodes. Blood is present in many of the sinuses, and macrophages containing many ingested erythrocytes are numerous. A few macrophages show a faint diffuse blue colour, or a few blue granules in the Prussian blue reaction.

Adrenals. The only lipoid demonstrable is in the form of some fine droplets in the glomerulosa, staining purplish blue with Nile blue sulphate.

Kidneys. In the majority of the glomeruli there is cubical to low columnar metaplasia of the capsular epithelium at the entrance of the tubule (Plate 14, Fig. 5). The cells of the first and second convoluted tubules and ascending limbs of Henle contain much diffuse dust-like and finely granular golden brown to dull brown pigment. These cells give a faint diffuse Prussian blue reaction and contain granules of haemosiderin, but not all the pigment granules, especially in the ascending limbs of Henle, are haemosiderin: they are possibly haematin or bile pigment. Some loose golden brown plugs in the secretory tubules are probably altered haemoglobin, and reddish brown casts in a few collecting tubules may be haemoglobin casts. There is pyknosis of the nuclei in a large proportion of the cells of the second convoluted tubules.

Scattered cells of the collecting tubules contain spherical cytoplasmic eosinophilic inclusion bodies. Their size varies from minute granules to bodies larger than the nucleus, and all gradations in size are sometimes present in one cell. The eosinophilic and hyaline appearance is most marked in the medium-sized inclusions. Some of the larger bodies appear to be agglomerations of smaller ones. Some cells contain a large number of inclusions, but cells containing one to twelve intermediate-sized inclusions are more common. They give a neutrophilic or basophilic reaction with Mann's eosin-methylene blue stain, and a minority only exhibit phloxinophilia in phloxin-tartarazine preparations (Lendrum, 1947).

Brain. After fixation in formol saline, the whole brain had a faint lemon yellow tinge macroscopically, but no part showed any special affinity for the pigment. In all six representative sections taken from different parts of the brain there is evidence of some

neuronal degeneration. Scattered nerve cells are shrunken with deeply staining cytoplasm and a very few others show chromatolysis. There is no cellular infiltration and the changes are diffuse and non-specific. No pigmentation is seen.

Case 2

Clinical observations

The foal was first noted to be lethargic when 15 hr. old. It died at 20 hr. when pallor, haemoglobinuria and icterus were well marked.

Serology

The foal's cells showed true 'auto-agglutination' in a saline medium after they had been washed free of their plasma. As would be expected, the other tests for sensitization were all positive.

The specificity and titre of the antibody or antibodies in the dam's serum are shown in Table 2. The agglutinin titre was practically as high as the titre of sensitizing antibody for many of the cells tested. This appears to be a very interesting antiserum as it contains antibodies for cells of horses which themselves have produced immune iso-antibodies. This fact shows very clearly that there is more than one antigen-antibody system which is capable of giving rise to this disease.

Haematology

The haematological examination was very difficult as the cells were strongly agglutinated, with the result that a red blood cell enumeration could not be performed. The haemoglobin content was 2 g./100 ml. and the white cell count 4400/cu.mm. No reticulocytes could be found.

Morbid anatomy

Macroscopic findings

The autopsy was performed 16 hr. after death. The foal weighed 50 kg. There was generalized icterus and very marked pallor of most of the organs. A small amount of fluid was present in the pericardial sac. The liver appeared normal and weighed 2·5 kg. The bile duct was patent. The spleen was grossly enlarged and weighed 2·3 kg. which is about four times the normal; the pulp was of 'blackberry jam' colour and consistency. The kidneys (140 g. each) and adrenals appeared normal.

The inner portion of the grey matter of the cerebral cortex had a slight yellow stain, but the cerebrospinal fluid was definitely icteric.

Microscopic findings

Bone marrow (mid-femur). The haemopoietic tissue is scanty in amount. Intermediate and late normoblasts are the most numerous nucleated red cells. There is much haemorrhage and very little haemosiderosis.

Liver. A moderate degree of congestion is present. It is rather uneven in distribution, but mainly central; elsewhere the sinusoids are compressed by liver cells. Many liver cells have swollen, pale, somewhat vacuolated cytoplasm and an ill-defined cell border. There is some irregularity and disruption of liver-cell columns and slight peripheral fatty changes. No bile pigment, haemosiderosis or erythrophagocytosis is seen.

Spleen. The pulp is extremely engorged with blood. A few widely separated Malpighian bodies and trabeculae appear to be floating in a sea of blood. The only other cells recognizable in the pulp are a few lymphocytes. No haemosiderosis is seen.

Adrenals. Some lipoid is present throughout the cortex but is most abundant as fine droplets in the glomerulosa and outer fasciculata, and as larger droplets in the innermost part of the cortex. Nile blue sulphate reveals granules with a similar distribution, which stain crimson red except in the outer glomerulosa where they are purplish blue.

Kidneys. An extension of the tubular type of epithelium into the capsule similar to that in case 1 is present in some glomeruli. No other significant abnormality is seen.

Brain. Macroscopically, after fixation, a pale lemon-yellow tinge was present at the upper end of the spinal cord and in the medulla oblongata, but nowhere else. The microscopic appearance is similar to that of case 1, except that chromatolysis is more conspicuous and there is engorgement of the small intra-cerebral arteries and capillaries with very occasional slight pericapillary haemorrhages.

Case 3

Clinical observations

This was the most acute case seen. The foal was born late one night and was found dead 8 hr. later. The most prominent signs were extreme pallor of the mucous membranes, haemoglobinuria and only very slight icterus.

Serology

The foal's cells did not show 'auto-agglutination' in a saline medium after washing them free of their plasma, but the direct antiglobulin-sensitization test showed them to be sensitized.

The reactions of the antibody in the dam's serum are shown in Table 2.

Haematology

No haematological examinations were carried out as the foal was found dead.

https://doi.org/10.1017/S0022172400036573 Published online by Cambridge University Press

Morbid anatomy

Macroscopic findings

The autopsy was carried out within 4 hr of death. The foal weighed 54 kg. There was extreme pallor with only slight icterus. No fluid was found in the body cavities. The liver (1·5 kg.) appeared normal, but the spleen (630 g.) was enlarged. The pulp was very dark but not of a 'jammy' consistency. The kidneys (140 g. each) and adrenals appeared normal.

Only a portion of the cerebral hemispheres of the brain were examined. There was no yellow staining.

Microscopic findings

Bone-marrow (mid-tibia) *spleen and adrenals.* The histological picture is almost identical with that seen in case 2.

Liver. There is slight congestion and fatty change. No haemosiderosis or other significant abnormality is seen.

Kidneys. No significant abnormality seen.

Brain. Unfortunately only the cerebral cortex was available and this was not fixed for 27 hr. after death. It shows occasional shrunken deeply staining ganglion cells.

CASE 4

Clinical observations

The foal appeared normal and thriving until the fourth day when slight pallor of the mouth was noted. On the morning of the fifth day icterus had developed and in the afternoon the foal was lethargic, was not sucking and could only just rise to its feet unaided. By nightfall it was prostrate and in the absence of facilities to prepare a transfusion of the dam's washed erythrocytes it was decided to give a transfusion of whole blood from a donor horse which appeared at the time to be compatible with the foal's blood, using a rough slide test in the field. The foal rallied and was given another transfusion 5 hr. later after which it rallied again; however, it died later during the night and subsequently it was found that the blood transfused reacted with the immune antibody in the dam's serum.

Serology

A blood sample was taken on the fifth day before giving the transfusion. The results of the different tests for *in vivo* iso-sensitization of these cells are shown in Table 3, and the reactions of the dam's serum are given in Table 2.

Haematology

The oxalated blood sample from this case did not reach the laboratory for about 16 hr. after venipuncture and the specimen was considerably haemolysed. For this reason and also the fact that the red cells showed 'auto-agglutination' in their own plasma the count of 510,000/cu.mm. obtained may in all probability not represent the true figure. The haemoglobin content of the sample was 3·2 g./100 ml. and the white cell count was 26,000/cu.mm.

Morbid anatomy

Macroscopic findings

The autopsy was carried out within 8 hr. of death. The foal weighed 54 kg. There was generalized icterus. Slight pulmonary oedema was present, but the lungs were well aerated. The liver appeared normal, and the bile duct was patent. The spleen was slightly turgid, the Malpighian corpuscles not being visible. The kidneys appeared normal but both adrenals were oedematous and showed patchy radial congestion of the cortex with areas of haemorrhage. The cerebrospinal fluid was bile stained but the brain substance was not.

Microscopic findings

Liver. A moderate degree of congestion is present which, on the whole, tends to be greatest in the centre of the lobules, but is irregular and focal in its distribution. The liver-cell columns are severely disrupted and many liver cells show degenerative changes of varying severity. A few focal necroses are present, usually in relation to a central vein; in them the liver cells have either disappeared or have shrunken granular eosinophilic cytoplasm. Infiltration with polymorphonuclear leucocytes in and around the necroses is only very slight.

The portal spaces appear larger than usual, but picro-Mallory stain shows that this is due to a separation of the connective tissue fibrils and not to any new formation of connective tissue. A similar condition is present in the walls and adventitia of the central veins and is possibly due to oedema. Iron pigment is present in moderate amounts, almost entirely as granules in Küpffer cells, but also in a more diffuse form in some liver cells. Bile thrombi in canaliculi are scanty. There is some erythrophagocytosis by Küpffer cells. No haemopoiesis seen.

Spleen. The pulp shows moderate to considerable engorgement. The trabeculae are broad owing to separation of the connective fibrils. The Malpighian bodies are small, ill defined and not rich in cells; degenerative changes and nuclear fragments are present in some. There is considerable haemosiderosis, for the most part as granules in fixed and free reticulum cells. Erythrophagocytosis is conspicuous.

Lymph nodes. There is slight congestion and sinus catarrh. Some macrophages in the sinusoids contain a pigment, pale yellowish brown in haematoxylin and eosin sections, but coarser and brownish black

in Prussian blue preparations counter stained with safranin.

Adrenals. Slight congestion is present in the outer fasciculata zone. Throughout the cortex there is considerable parenchymatous degeneration, with separation of cell columns and individual cells. The cells of the glomerulosa and outer fasciculata and to a lesser extent of the inner border of the cortex, contain fine to medium-sized bright orange lipoid droplets in sections stained with Scharlach R. Nile blue sulphate shows a similar distribution of dark purple-blue granules.

Kidneys. Slight congestion is present in the medulla and some glomeruli, and there is severe degeneration of the first convoluted tubules. In the majority of glomeruli there is a crescent in contact with the capsular basement membrane similar in appearance to the degenerate cells of the first convoluted tubules, and in glomeruli, sectioned in the appropriate plane, continuous with these cells. They almost certainly represent an extension of the tubular type of epithelium into the capsule as seen in cases 1 and 2. Hyaline thrombi are present in some glomeruli. Nuclear fragments are conspicuous in the intertubular connective tissue. No bile pigment or haemosiderin seen.

Case 5

Clinical observations

This was a subacute case in which the clinical signs were marked only on the fifth day. The cardinal signs were lethargy and icterus. There was no haemoglobinuria. The cardiovascular signs were of moderate severity only. Despite the very low values found in the haematological examinations, on the appraisal of the clinical signs it was decided not to carry out transfusion therapy unless the animal's condition became worse. The animal made a spontaneous and, as far as can be determined 4 months later, complete recovery without specific therapy.

Serology

The foal's red cells on the fifth day were shown to be sensitized by the direct antiglobulin-sensitization test. The results obtained with the other tests for sensitization are shown in Table 3.

The reactions of the immune iso-antibody in the dam's serum are given in Table 2. No free sensitizing antibody could be found in the foal's plasma as was demonstrated in the plasma of the foal of case 1.

Haematology

The haemoglobin and red and white cell counts which were carried out over the period of recovery are recorded in Fig. 2. Curiously, in view of the benign course of the disease, the haemoglobin and erythrocyte levels were initially even lower than those of case 1, although this may well be due to the different stages of the disease at which the bloods were examined—the fifth and second day respectively.

In this case also, the blood examined on the fifth day showed 1% reticulocytes, although in no subsequent specimen was more than 0·2% observed. The erythrocytes showed some degree of macrocytosis and polychromatophilia, but normoblasts were never found in the peripheral blood. Although most of the early blood specimens showed considerable icterus, free haemoglobin was rarely present in the plasma. After a stationary period of approximately 4 days (the fifth to ninth day of life) the haemoglobin and red-cell levels rose gradually and steadily without the aid of transfusion.

Case 6

Clinical observations

This was another subacute case whose clinical course was practically identical with that of case 5. Specific transfusion therapy was withheld for the same reasons as in the last case. The foal made a spontaneous and complete recovery.

Serology

Once again the foal's red cells were shown to be sensitized by the direct antiglobulin-sensitization test (Table 3). Diamond's albumin tube test performed on unwashed cells deposited in their own plasma was positive although the reaction was not strong. After the cells had been washed in saline there was no 'auto-agglutination' in either a saline or albumin medium.

Table 2 records the reactions and the respective titres of the antibodies in the dam's serum.

Haematology

This foal showed less anaemia than the previous case and the haemoglobin and erythrocyte levels rose more rapidly, also without the aid of transfusion. The graph of the blood response in comparison with that of case 5 is shown in Fig. 2. The highest reticulocyte response observed was 0·4% on the eighth day. Although mildly icteric, the plasma never contained free haemoglobin.

Control cases 7 and 8

It was not possible to obtain blood samples from normal thoroughbred foals of various ages to control the serology and haematology described above, nor were any casualties available to supply normal material to control the morbid histology. However, two jaundiced foals in which iso-immunization was

https://doi.org/10.1017/S0022172400036573 Published online by Cambridge University Press

not concerned in the aetiology, supplied us with control samples and material of a particular type.

Clinical observations

Both these foals showed lassitude and icterus within 24 hr. of birth, but no signs of acute anaemia. Case 7 was affected with severe lassitude at birth, and icterus was definitely present 18 hr. later. The foal died when 24 hr. old. Both the clinical course and autopsy findings were suggestive of foal septicaemia with generalized icterus, possibly due to *Bacterium equirulis*.

Case 8 was normal for 18 hr. after birth when it became lethargic and at 24 hr. icterus was marked, but without any pallor of mucous membranes or haemoglobinuria. The foal died when 36 hr. old. The autopsy findings were suggestive of a foal septicaemia and a coliform organism was obtained in pure culture from the parenchymatous organs. Except in this case no bacteriological examinations were carried out in these investigations.

Serology

The direct antiglobulin sensitization test was negative in both these cases showing that the foals' cells were not sensitized by maternal antibody.

The serum of the dam of foal 7 was examined and no immune iso-antibodies could be demonstrated (Table 2). The other dam's serum was not examined.

In both these cases the serological findings excluded iso-immunization as the cause of the diseased state.

Haematology

Complete examinations were impossible in case 7 as the foal was dead when first seen by us. No nucleated erythrocytes were seen in peripheral blood smears, nor was there any evidence of erythropoietic hyperplasia in bone marrow smears.

Unfortunately no enumerations were carried out on the blood of case 8 either, although it was noted to have a haematocrit of about 40–45 %.

Morbid anatomy

Only the morbid histological findings are recorded here. Besides foals 7 and 8 another foal dying at 36 hr. with the clinical condition known as 'dummies' supplied another source of control material for histological examination.

The bone marrow (mid-femur) (Plate 14, Fig. 2) in these three cases contains very little haemopoietic tissue and the cellular pattern is similar to that of cases 2 and 3. The livers do not contain any bile pigment or haemosiderin, nor are there any necroses. In the spleens the Malpighian bodies are large, and there is no haemosiderosis. The lymph nodes contain pigment similar to that in cases 1 and 4. The lipoid content of the adrenals and its distribution resemble cases 2 and 3. There is no cubical metaplasia of the glomerular capsular epithelium or any other significant abnormality present in the kidneys. No erythrophagocytosis was seen in any of the organs examined.

In this material there is no evidence of any severe blood destruction or compensatory blood regeneration, and no specific degenerative changes in the liver or other organs which could not be accounted for by the septicaemic condition.

IV. DISCUSSION

Although this is only a preliminary study of the haemolytic syndrome in newborn thoroughbred foals due to iso-immunization of pregnancy it is probably the first in which the serology has been linked with the other pathological and clinical findings. It follows on the pioneer study of Caroli and Bessis on the same condition in newborn mules. A much more comprehensive piece of work is required to verify the various points of interest which have been demonstrated.

A fully detailed report of the clinical findings will be given elsewhere. It appears that the clinical manifestations in the foal, resulting from iso-immunization of pregnancy, constitute in themselves a definite clinical syndrome. Cases 7 and 8, however, serve to illustrate a condition of septicaemic jaundice in newborn foals which can be confused clinically with the haemolytic disease due to iso-immunization of pregnancy.

Modifications of the serological procedures used in the diagnosis of haemolytic disease in newborn human infants afford the quickest and most sure methods of diagnosing this disease of foals. We have found the examination of the foal's red cells for *in vivo* iso-sensitization by the direct antiglobulin-sensitization test the most reliable method of diagnosis. The findings of this test may be confirmed by the examination of the dam's serum for immune iso-antibodies for horse red cells of certain genotypes.

Diamond's albumin tube test, as we used it to detect sensitization of foals' cells, did not give decisive easily read results, as some sensitized cells were only weakly agglutinated and cells of control horses did not give absolutely negative results. With further work, however, to find the optimal way of performing the test, it is quite possible that the method would be quite suitable. 'Auto-agglutination' of red cells in the animal's own plasma cannot be used for diagnosis, as the cells of many normal horses also exhibit a non-specific clumping phenomenon. The serological procedures for the diagnosis of the disease in newborn thoroughbred foals have also been found suitable for the diagnosis of the condition in newborn mules.

The specificities of the antibodies in the dams' sera have still to be worked out. These sera have been preserved and will be most valuable in any eventual investigation of the red-cell genotypes of horses.

It is generally accepted that nearly all the antibody transferred passively from the mare to her offspring passes via the colostrum, except perhaps in the case of a hyperimmunized mare in which case placental transmission may also play a part (Lémetayer, Nicol, Jacob, Girard & Corvazier, 1946). This conclusion is of great importance in the understanding and treatment of the disease. In none of these cases were we able to examine the foal's cells for *in vivo* sensitization before it had sucked the dam, but we hope to be able to do this next year, when we shall know the animals in whose foals we may expect a recurrence of the disease. If it is then found that all the antibody usually reaches the foal via the colostrum, it would provide very strong grounds for nursing the foal either on another mare, if available, or on modified cow's milk. This was not done in any of the cases reported this year, as theveterinary surgeons, under whose care these cases were, considered that in the light of our present incomplete knowledge the disadvantages of such a procedure might well outweigh the possible advantages. However, further experience in the field will decide the best procedure to adopt. It will be necessary to carry out some experiments, such as have been done on the calf, to ascertain up to what age the foal is able to absorb antibodies in their native state from the alimentary canal. It is of interest that in case 5, in which the disease in the foal was relatively mild, the mare was noted to be losing colostrum from the udder for 2 days before foaling; and this may have had some influence on the spontaneous recovery of this case.

From observations on the haematological findings it is clear that this condition in foals cannot be called an erythroblastosis, since erythroblasts were never seen in the peripheral circulation. It also seems remarkable that foals 5 and 6 were clinically so mildly affected despite their pronounced anaemia. The very low reticulocyte counts observed, especially in these two cases, are of interest considering the active regeneration which must have been going on.

The morbid histological findings furnish much material for comment. The hyperplastic marrow in case 1, which survived 13 days, with its high proportion of the more immature cells of the erythroblast series, is the normal response of the bone marrow in severe anaemia. The absence of hyperplasia in cases 2 and 3, both of which died within 24 hr. of birth, suggests that the duration of the anaemia was too short for any such reaction to occur. The absence of extramedullary haemopoiesis, even in case 1, is at first sight surprising when compared with the striking picture in human infants. However, the gestation period in horses is 11 months and it seems possible that, in the case of the horse, haemopoiesis may be entirely intramedullary for some time before birth. If this is so, it would seem reasonable to suppose that a much greater stimulus is required for haemopoiesis to reappear in the liver or any other tissue of the foal than is necessary in the infant in whose liver it normally persists for 3–5 days after birth.

In the livers of cases 1 and 4, apart from signs of a severe haemolytic process, namely erythrophagocytosis, haemosiderosis and jaundice, there is evidence of damage to liver cells, i.e. diffuse degeneration, focal necroses, and in case 1 an early cirrhosis. The latter resembles early human toxic cirrhosis and there is no evidence that obstruction in the biliary passages by inspissated bile was a factor in its causation, as has been suggested in some human cases (Lightwood, 1943; Skelton & Tovey, 1945; Hawksley & Lightwood, 1934). The jaundice was probably partly toxic in origin as bile thrombi in canaliculi are not a feature of haemolytic jaundice uncomplicated by damage to liver cells (Gilmour, 1944). The paucity of bile thrombi in case 4 may possibly be explained by the complete separation of the majority of liver cells from each other. The absence of any specific change in the livers of cases 2 and 3, apart from a general unhealthy appearance in the former, may be due to the short duration of the illness.

An acute haemolytic process of short duration could have produced the extreme engorgement without haemosiderosis of the spleens in cases 2 and 3. The erythrophagocytosis and haemosiderosis in cases 1 and 4 are the expected consequences of a less severe and more prolonged destruction of blood cells.

The appearance of the abnormal Malpighian bodies in case 1 suggests hyalinization following a severe relatively slow degeneration, but this is by no means certain. Similar but less extensive changes are seen not infrequently in infants dying from a variety of diseases.

The extension of the tubular type of epithelium into the glomerular capsules in the kidneys of cases 1, 2 and 4 is interesting. (The absence of this finding in case 3 may be due to the extremely short duration of the disease in this case.) It has been reported in acute myohaemoglobinuria (Bywaters & Dible, 1943) and in traumatic anuria (Bywaters & Dible, 1942), and may well be connected with the excretion of large quantities of abnormal pigments, as haemoglobinuria was present in all three cases. The cytoplasmic inclusions seen in scattered cells of the collecting tubules in case 1 are probably an incidental finding. They do not agree with the description of inclusions, almost certainly of virus origin, found in the organs of infants and recently reported in two cases of haemolytic disease by Cappell &

McFarlane (1947), but this does not exclude the possibility, which their appearance in some ways suggests, that they are another type of virus.

We ourselves do not know the amount and distribution of lipoid in the adrenal cortex of a normal foal, and so it is difficult to assess the significance of the difference in this respect between cases 2 and 3 and cases 1 and 4. Since the picture in the former and in the 'controls' (also only living 36 hr. or less) is similar, it is probable that the relative scarcity of lipoid in cases 1 and 4 is a non-specific response to illness.

The neuronal degeneration observed in the brains appears to be entirely non-specific; the combined effects of severe anaemia and post-mortem change are sufficient to account for it.

Erythrophagocytosis is frequently seen in infants for a few days after birth, but would be abnormal at 6 and 13 days, the ages of occurrence in cases 4 and 1; its pathological nature is confirmed by its absence in the two younger foals and in the controls.

From the experience of Caroli and Bessis in France on the condition in newborn mules, and our experience in this country of the condition in newborn thoroughbred foals, we can suggest possible lines of specific treatment of the disease which would be practicable in the field. Certain subacute cases, such as cases 5 and 6, are able to recover without any specific transfusion therapy, but with careful nursing only. It may well be that the clinical state of the foal should form the criterion of whether a transfusion should be given or not. Such a decision would be easier if the question as to whether the mare should be allowed to suckle the foal or not were decided; for until we know for how long the foal is capable of absorbing protein in its native state from the alimentary canal, there is the consideration that the foal will be receiving more and more antibody for many days after birth and thus presumably worsening its chances of spontaneous recovery.

Should a transfusion be considered necessary, the best choice of a donor would not be an easy matter, owing to our present incomplete knowledge of the blood groups of horses. If only one, or at the most two, transfusions were needed, it would only be necessary to find a horse whose blood did not react with the immune antibody in the serum of the dam and foal. On the other hand, if many transfusions were needed, it would be necessary to have the donor's blood completely compatible with that of the foal, for although the first or second transfusions would probably not cause a reaction, owing to the low normal iso-agglutinins even in adult horses, it is possible that the foal would become actively sensitized against the transfused cells with the result that, even if there was no transfusion reaction, the transfused cells would not be maintained. We think this probably happened in case 1.

Exsanguino-transfusion would seem to be the best and most practical method of specific transfusion therapy. This has been the procedure adopted by Bessis and Caroli for the treatment of haemolytic disease in newborn mules. If blood of the correct group were used, this method should be highly satisfactory and practical, for not only does the procedure introduce new healthy unsensitized red cells but it also removes 60–80 % (depending on the amounts transfused and exsanguinated) of the foal's serum which may contain a high titre of maternal antibody still unabsorbed. A single exsanguino-transfusion may be quite sufficient to set the animal on the road to recovery, which would be a great consideration in veterinary practice. The technique of performing exsanguino-transfusions in mule foals is being published shortly by Bessis.

Even with exsanguino-transfusion the question of choosing the best donor blood is all important. Before we are in a position to do this, an investigation is necessary to study the blood groups of horses, and in particular to study the specificities of the immune iso-antibodies produced by the dams as a result of iso-immunization of pregnancy. We have six such sera at the moment, but it appears that most of these contain mixtures of several antibodies.

Once it has been found which are the most common antigen-antibody systems responsible for the disease, certain horses in a breeding district could be examined and those whose cells do not react with these antibodies (i.e. *Rh* negative by analogy with the human disease) might, with the owners' consent, be considered donor horses. Such known donor horses could then be called on in an emergency to donate blood for an exsanguino-transfusion of a newborn foal suffering from this disease.

It will obviously be necessary to follow up both spontaneously cured and treated cases for any sequelae which may develop only later in life.

We feel that a continued and more complete investigation of the condition would not only be of interest to the veterinary profession, but also, as a study in comparative pathology, would be of interest to workers engaged in the study of the human disease. The study of the blood groups in the horse is a problem in itself worthy of some attention.

V. SUMMARY AND CONCLUSIONS

1. Six cases of haemolytic disease in newborn foals apparently due to iso-immunization of pregnancy are described from their clinical, serological, haematological and pathological aspects. The findings agree closely with those seen in the same disease in newborn mules.

2. The disease may be diagnosed serologically by demonstrating the *in vivo* iso-sensitization of the

https://doi.org/10.1017/S0022172400036573 Published online by Cambridge University Press

foal's red cells by means of the direct antiglobulin-sensitization test. It is quite possible that after further investigation a modification of Diamond's albumin tube test may also be of value. Immune anti-red cell iso-antibodies of more than one specificity may be demonstrated in the dams' sera.

3. Despite the acute haemolytic process, very few reticulocytes and no erythroblasts are seen in the peripheral blood.

4. The morbid histology of the tissues of foals dying with the disease exhibits various points of interest which are discussed.

5. The clinical aspect is not described in this paper in any detail. However, a programme is suggested which would enable a successful exsanguino-transfusion of compatible blood to be carried out at very short notice. At present this seems to be the logical and most practical method of specific treatment.

The authors would like to express their thanks to Dr Walker of the Regional Blood Transfusion Laboratory, Cambridge, for his co-operation in lending us transfusion apparatus.

They would also like to express their thanks to Mr Patman of the Department of Pathology, Cambridge, for taking the photomicrographs.

ADDENDUM

Since this paper has been in press we have obtained a sample of blood from the sires of cases 2, 3, 5 and 6. In each case the red blood cells contained an antigen or antigens which reacted with the serum of the respective dam.

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EXPLANATION OF PLATE 14

Fig. 1. Case 1. Bone-marrow (mid-femur). Excessive haemopoiesis. Haematoxylin and eosin. × 450.

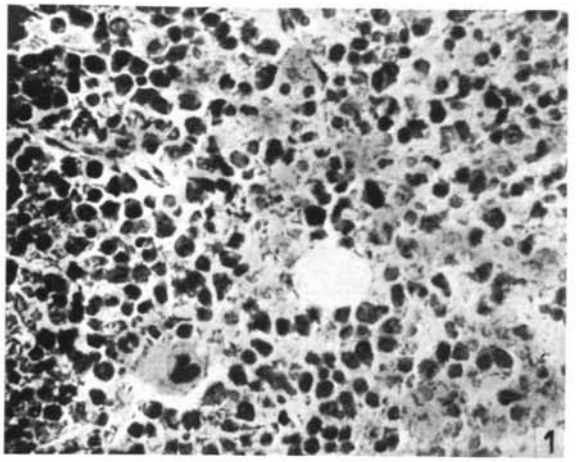
Fig. 2. Case 7. Bone-marrow (mid-femur). Normal content of haemopoietic tissue. Haematoxylin and eosin. × 450.

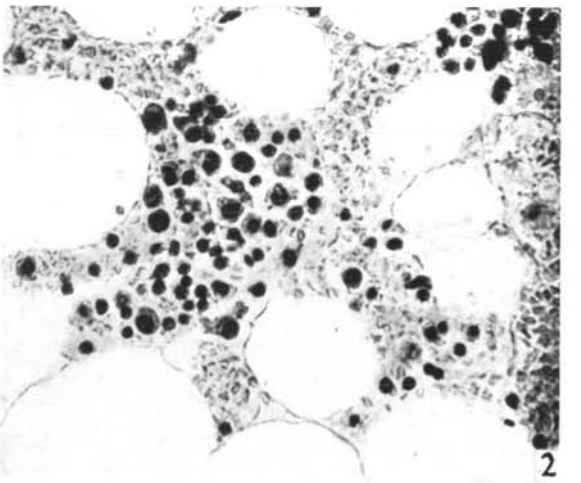
Fig. 3. Case 1. Liver. Early cirrhosis. Haematoxylin and eosin. × 92.

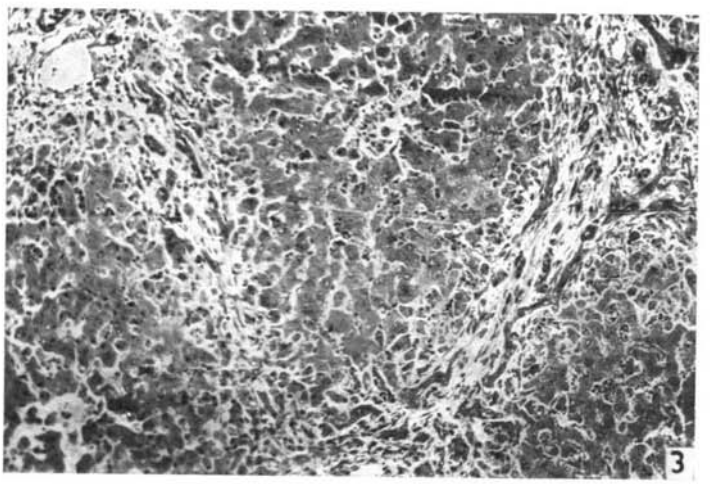
Fig. 4. Case 1. Liver. High power of portal tract. Pseudo-bile canaliculi and fibroblasts in portal tract, and swollen pigment containing macrophages in sinusoids. Haematoxylin and eosin. × 410.

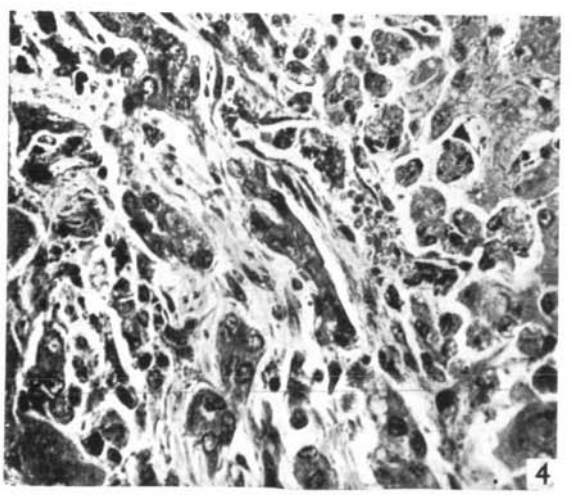
Fig. 5. Case 1. Kidney. Extension of tubular type of epithelium into the glomerular capsule. Haematoxylin and eosin. × 370.

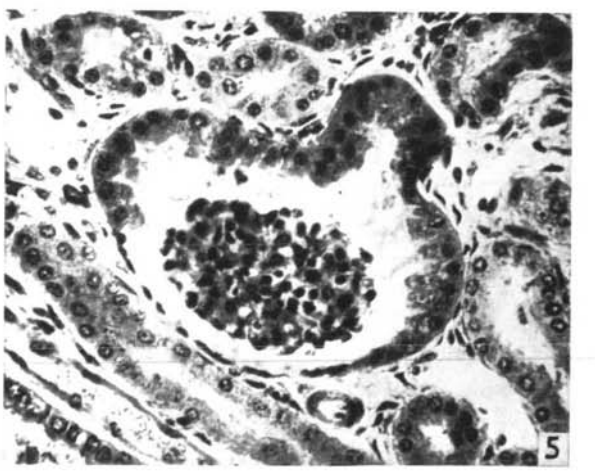
(*MS. received for publication* 12. VIII. 48.—Ed.)











https://doi.org/10.1017/S0022172400036573 Published online by Cambridge University Press