Studies on the Neurotropic Virus of Horsesickness. VII.—Transmitted Immunity.

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In the course of routine investigations into the efficacy of the neurotropic horsesickness vaccine issued from Onderstepoort, we have been impressed by the rather heavy mortality amongst immunized yearlings. As an instance we may quote the experience on a farm in the Naboomspruit District of the Northern Transvaal. This farm is situated in the low-lying marshy valley of the Nyl and is notorious as a " bad horsesickness farm ". The owner runs some 40 bigframed nondescript mares which are being bred to a crossbred Percheron stallion for the production of a light type of draught animal. There is practically no stabling accommodation, the troop of horses being allowed to run day and night. Annual immunization against horsesickness is carried out under our immediate control and steps are taken to obtain an accurate diagnosis in all cases of death. In May, 1937, at the conclusion of a severe horsesickness season, it was reported that, of 40 immunized horses, 8 (20 per cent.) had died of horsesickness and an additional 4 had shown clinical symptons of infection but had recovered. At first sight these figures appeared to be discouraging but a more careful survey showed that of the 8 fatal cases 6 were yearlings out of immunized dams and only 2 were adult animals; the 4 animals that recovered consisted of 2 aged mares and 2 geldings. The yearlings that died had been inoculated when they were between 3 and 5 months old. Again, at the end of the 1938 season it was found that, out of 68 animals treated, 16 had died (23·5 per cent.). Examination of the figures showed that, of the 16 dead animals, no fewer than 13 were yearlings and that only one of the adult horses had died of horsesickness uncomplicated with biliary fever.

To throw some light upon these aspects of the problem of immunization against horsesickness we decided to carry out a detailed investigation into the possible transference of immunity from dam to offspring. As sufficient brood mares to unable such a study to be carried out are not maintained at Onderstepoort, we were fortunate in being able to avail ourselves of the facilities placed at our disposal by the Commissioner of the South African Police, Major-General I. P. de Villiers, through his Veterinary Staff Officer, Major D. D. Morton M.R.C.V.S. in charge of the Police Stud Farm, Kimberley. Without this ready co-operation, which we take this opportunity of acknowledging, it would have been exceedingly difficult to initiate the work which forms the basis of this report.

The general scheme of the investigation was to follow the variations in immunity of a group of foals out of immune dams from the time of birth till the age of about 12 months. This could be done quantitatively by applying the intracerebral protection test (Alexander 1935) to an estimation of the antibody content of the serum from time to time. Soon after the work began results of great interest were obtained, necessitating a considerable expansion of the scope of the investigation.

TECHNIQUE.

The technique of the intracerebral protection test in mice for the quantitative estimation of the antibody content of immune serum has been described in detail (Alexander 1935, 1936). In the series of experiments to be recorded the stock virus antigen was diluted in each instance so that unit volume of serum was required to neutralize 100 minimal lethal doses of virus. As soon as a stock antigen showed signs of "going off" a fresh emulsion was made up and standarddized; usually an antigen could be relied upon to maintain its titre for a period of about 2 months when stored in the refrigerator at 4°C.

To avoid any possibility of error, tests with each sample of serum were always run in duplicate on different days. If by any chance the results did not agree within the limits of experimental error, usually three and if necessary, five repeat tests were set up. It must be emphasized however that almost invariably the results of duplicate tests were readily repeatable.

An obstacle to the development of work of this type in the past has been the difficulty experienced in obtaining suitable samples of serum from the field. Our experience has been that samples of blood forwarded by post or by rail from the field invariably arrived in such a haemolysed or decomposed condition that the serum was quite unsuitable for *in vitro* neutralization tests. However, adoption of the method described by De Kock, Robinson, and Parkin (1939) for the collection of serum for complement-fixation tests in the diagnosis of dourine has overcome this difficulty, i.e. bleeding into sterile bottles containing sufficient boracic acid solution to produce a final concentration of 1 per cent. boracic acid. In every instance an adequate amount of clear serum was collected. A comprehensive series of preliminary experiments was run to determine whether this concentration of boracic acid would have any effect upon the results of neutralization tests. It was found that no effect whatever could be detected.

EXPERIMENTAL PROCEDURE.

Five pregnant mares were selected at random from a troop which had been inoculated twice with routine neurotropic horsesickness vaccine. The last injection had been given approximately 12 months previously. These mares were placed in loose boxes and watched day and night up to the time of foaling. As soon as the foals were born they were rubbed down and dried to stimulate circulation and a sample of blood was drawn from the jugular vein usually within 20

minutes of birth, i.e. before the foal was allowed to suck. Next day, i.e. from 16 to 30 hours after birth and after the foal had ingested some milk, a second sample of blood was taken. At this time a sample of blood was withdrawn from the dam as well. Further samples of blood were obtained from the foals at the times indicated in Tables 1 and 2; during this period the foals were allowed to run with the dams. The results of the serum-virus neutralization tests are given below. Although the vaccine used for immunization contained 6 antigenically different strains of virus, tests were carried out against only two of these strains, viz., those labelled Vryheid and 449. These two strains were selected because they appear to contain a minimum of common antigen.

TABLE 1.

Neutralization of Virus Vryheid by Foal and Dam Sera.

Mare.		Age of Foals and Titre of Serum.						
Number.	Titre of Serum.	½-Hour.	16 to 30 Hours.	20 Days.	75 Days.	125 Days.	175 Days.	325 Days.
42.........	$>1/2$	$>1/2$	$>1/2$	$>1/2$	$>1/2$	$>1/2$	$>1/2$	$>1/2$
85.........	$1/128$	$>1/2$	$1/256$	$1/128$	$1/64$	$1/4$	$>1/2$	$>1/2$
146.........	$1/32$	$>1/2$	$1/32$	$1/8$	$1/2$	$>1/2$	$>1/2$	$>1/2$
275.........	$1/64$	$1/2$	$1/128$	$1/32$	$1/32$	$1/4$	$>1/2$	$>1/2$
322.........	$>1/2$	$>1/2$	$1/2$	$>1/2$	$>1/2$	$>1/2$	$>1/2$	$>1/2$

TABLE 2.

Neutralization of Virus 449 by Foal and Dam Sera.

Mare.		Age of Foals and Titre of Serum.						
Number.	Titre of Serum.	½-Hour.	16 to 30 Hours.	20 Days.	75 Days.	125 Days.	175 Days.	325 Days.
42.........	$1/64$	$>1/2$	$1/128$	$1/64$	$1/8$	$>1/2$	$>1/2$	$>1/2$
85.........	$1/256$	$>1/2$	$1/512$	$1/512$	$1/256$	$1/8$	$1/4$	$>1/2$
146.........	$1/256$	$>1/2$	$1/256$	$1/64$	$1/16$	$1/2$	$>1/2$	$>1/2$
275.........	$1/128$	$>1/2$	$1/512$	$1/128$	$1/16$	$1/2$	$1/2$	$>1/2$
322.........	$1/64$	$>1/2$	$1/64$	$1/32$	$1/4$	$>1/2$	$>1/2$	$>1/2$

NOTE.—When the foals were 175 days old, i.e. at the time of the 6th bleeding, each received an injection of routine vaccine. The efficacy of this batch of vaccine was controlled by injecting it into a susceptible horse, 22000, at Onderstepoort. This horse developed a satisfactory immunity as shown by the subsequent development of high-titre antibodies (*cf.* Table 6, page 26).

Results.—This experiment yielded a number of most interesting results some of which are the subject of further detailed investigation at the present time.

Without exception no trace of antibodies could be demonstrated in the serum of foals immediately after birth. From this it is apparent that *in utero* the placenta is an effective barrier to the transfer of antibodies to the virus of horsesickness from the maternal to the foetal circulation. Within a few hours of first sucking, the antibody titre of the foal serum rose to a point at least equal to that of the dam and in three out of the five cases to a higher titre (cf. foals from dams 42, 85 and 275). It is reasonable to assume that the presence of the antibodies resulted from the ingestion of the colostral milk, but there is no direct evidence in support of this assumption; data will be collected only when a number of obvious practical difficulties in the way of such an investigation have been overcome. This immunity which is undoubtedly passive, gradually declined, indicating that the high concentration of antibodies did not persist in the mare's milk or if they were present were not absorbed unchanged. At the age of 175 days traces of antibody only were detectable, yet the foals did not develop an active immunity following the injection of vaccine. Apparantly sufficient antibodies were present to neutralize the virus contained in the vaccine though the intracerebral protection test was insufficiently delicate to demonstrate their presence.

As regards the immunity of the mares it will be seen that all had developed antibodies to reasonably high titre against strain 449. The titres varied considerably (from 1/64 to 1/256), but this variation calls for no comment. On the other hand two mares (42 and 322) had developed no demonstrable antibodies to the antigenically dissimilar strain Vryheid in spite of the fact that all the animals had received the same treatment viz. two annual injections of polyvalent vaccine containing 6 virus strains. This finding does not agree with the report on the antigenic response of horses to simultaneous trivalent immunization (Alexander 1936) and is being investigated further.

The acquisition of antibodies by the foals was strictly specific in that foals out of mares whose serum contained antibodies against strain 449 only in turn developed antibodies against that strain alone. The higher the antibody content of the dam's serum, the higher was the titre of the foal serum on the day after birth. Finally it is seen that the length of time demonstrable antibodies persisted in the foal serum was correlated with the titre of the serum of the dam at the time of the foal's birth.

As it appeared to be important to determine approximately the earliest age at which it is possible to produce an active durable immunity in foals from immune dams by the injection of neurotropic vaccine an additional four foals were selected viz. 311 when 76 days old, 264 when 87, B37 when 173, and 137 when 190 days old. A sample of blood was taken from each and then a subcutaneous injection of 10 c.c. of an emulsion containing at least 1,000 M.I.D. of strains 449 and Vryheid was given. The virus titre of this experimental vaccine was determined by intracerebral injection of

mice on the assumption that if 0·05 c.c. contains 1 M.I.D. for a mouse then 10 c.c. contains 1 M.I.D. for a horse (Alexander, Neitz, and Du Toit 1936). After an interval of 74 days, samples of serum were again collected and the foals were given routine vaccine. A final sample of serum was obtained 112 days later. The results of the intracerebral protection tests with these sera are given in Tables 3 and 4. No tests were carried out on serum from the dams.

TABLE 3.

Neutralization of Virus Vryheid by Foal Sera.

Foal.	Age.	Titre.	Interval.	Titre.	Interval.	Titre.
311....................	76 days	$\frac{1}{2}$	74 days	$> \frac{1}{8}$	Foal died	—
264....................	87 days	$\frac{1}{8}$	74 days	$> \frac{1}{8}$	112 days	$> \frac{1}{2}$
B37....................	173 days	$\frac{1}{8}$	74 days	$\frac{1}{2}$	112 days	$> \frac{1}{2}$
137....................	190 days	$> \frac{1}{2}$	74 days	$> \frac{1}{2}$	112 days	$> \frac{1}{2}$

TABLE 4.

Neutralization of Virus Vryheid by Foal Sera.

Foal.	Age.	Titre.	Interval.	Titre.	Interval.	Titre.
311....................	76 days	$\frac{1}{4}$	74 days	$> \frac{1}{2}$	Foal died	—
264....................	87 days	$> \frac{1}{2}$	74 days	$> \frac{1}{8}$	112 days	$\frac{1}{4}$
B37....................	173 days	$> \frac{1}{2}$	74 days	$> \frac{1}{2}$	112 days	$> \frac{1}{2}$
137....................	190 days	$> \frac{1}{2}$	74 days	$> \frac{1}{2}$	112 days	$> \frac{1}{2}$

Result.—Four foals from immunized dams and between the ages of 76 and 190 days did not develop an active immunity following the injection of 1,000 M.I.D. of each of strains 449 and Vryheid. Only two strains of virus were included in the emulsion injected so as minimize the possibility of mutual interference by a plurality of virus strains. The oldest foal in the experiment had reached an age of almost 9 months (264 days) when it received the normal dose of routine vaccine. Neither it nor the two younger survivors developed immunity.

It is necessary to direct attention to the fact that the youngest surviving foal (264) which received polyvalent vaccine at the age of 161 days at a time when no detectable antibodies were present in the serum, subsequently was found to have an antibody titre of 1 : 4 against strain 449 though less than 1 : 2 against strain Vryheid. The significance of this low antibody titre is not entirely clear but we believe it to be related to the presence of high titre antibodies against one of the other closely related strains incorporated in the polyvalent vaccine. Insufficient serum was available to investigate this point.

At this stage an opportunity presented itself of applying a direct *in vivo* immunity test to two foals from immune dams amongst the troop of horses at Onderstepoort. Since the original virulent pantropic strains 449 and Vryheid had inadvertently been lost, strain O was used. This strain which has been passaged for 201 generations through horses is exceedingly virulent. In susceptible horses it can be relied upon to cause 100 per cent. mortality, death occurring usually not later than the 6th or 7th day. The histories of the 4 mares and foals included in this experiment are summarized below :—

Mare 20881.

    6. 3.1934.   Fully susceptible. Immunized with routine quadrivalent vaccine (449, 464, O and Vryheid).

   18. 3.1935.   Received 5 c.c. of Vryheid pantropic virus. Reacted very severely but recovered.

   10. 2.1938.   Immunized with routine polyvalent vaccine (449, 464, O, Vryheid, K.A. and O.D.).

   24. 7.1939.   Do.

   13.10.1939.   Gave birth to foal 22180.

   23.11.1939.   Blood collected; 5 c.c. O virus generation 201 intravenously. No reaction.

Foal 22180.

   13.10.1939.   Born of mare 20881.

   22.11.1939.   Blood collected; intravenously 5 c.c. O virus generation 201. No reaction.

   1. 2.1940.   Do.   Do.

   25. 2.1940.   Died as a result of an accident; serum could not be collected.

Mare 21159.

   8.10.1937.   Fully susceptible; received 10 c.c. quadrivalent vaccine; mild reaction.

   10. 9.1939.   Gave birth to foal 22167.

   5.12.1939.   Blood collected; received 5 c.c. O virus generation 201. No reaction.

Foal 22167.

   10. 9.1939.   Born of mare 21159.

   1.12.1939.   Blood collected; received 5 c.c. O virus generation 201. No reaction.

   6. 5.1940.   Blood collected; received 5 c.c. O virus generation 202. Reacted after a lengthened incubation period of 5 days and died on 9th day.

The virulence of the O virus was controlled by injecting it into a susceptible horse, 21558, on 17.12.39. This horse reacted after an incubation period of 3 days and died on the 6th day.

The results of the intracerebral mouse protection tests carried out with the sera collected, together with the results of the *in vivo* immunity tests are shown in Table 5. It is necessary to point out that in the neutralization tests, the antigen used was adjusted so that unit volume of serum was required to neutralize 50 M.I.D. of virus and not 100 M.I.D. as in the case of the other strains.

TABLE 5.

In vivo *and* in vitro *Immunity Tests of Foals from Immune Dams.*

Animal.	Age in Days.	Serum Titre.	Immunity Test.	Age in Days.	Serum Titre.	Immunity Test.	Age in Days.	Serum Titre.	Immunity Test.
Mare 20881..	—	$<^1/_{256}$	NR	—	—	—	—	—	—
Foal 22180..	40	$^1/_{128}$	NR	112	$^1/_{32}$	NR	—	—	—
Mare 21159..	—	$<^1/_{1024}$	NR	—	—	—	—	—	—
Foal 22167..	82	$<^1/_{256}$	NR	151	$^1/_8$	—	239	$>^1/_2$	R†

NR = No reaction.
R† = Reacted and died of horsesickness.

Results.—In the case of both mares the intracerebral mouse protection test showed that the serum contained antibodies to a **high** titre, in fact in neither case was the end point of titration reached: both mares were solidly immune to the intravenous injection of virulent pantropic virus. It is interesting to note that whereas the first mare (20881) had received repeated injections of vaccine, the second (21159) received a single injection of vaccine and yet approximately 2 years later the antibody content of the serum was high. During the two horsesickness seasons after immunization this mare was stabled under conditions where no cases of horsesickness occurred so that it is highly improbable that the immunity was augmented as a result of natural infection.

When 40 days old the first foal (22180) had a serum titre of 1/128 and was immune to a test injection of virulent virus; 72 days later the serum titre had decreased to 1/32, i.e., at approximately the rate to be anticipated from the results obtained with other foals from immune dams (*cf.* Tables 2 and 3). Unfortunately the experiment was terminated by an accident. The second foal was given an immunity test when 82 days old. At this time the antibody titre was high (more than 1/256) and as was to be expected the foal did not react to the *in vivo* test. Neutralization tests carried out with serum obtained 69 days later (age 5 months) showed that the antibody titre had decreased at the anticipated rate to 1 : 8; no *in vivo* immunity test was applied. When the foal was 8 months old no antibodies could be detected in the serum. On immunity test the foal reacted and died. It is necessary to emphasize, however, that the period of incubation was prolonged and that the course of the disease was atypical. Considered in the light of a wide experience

with the serum-virus-simultaneous method of immunization the impression was created that there existed a slight immunity just unable to cope with the virulent virus injected; this would be analogous to an inadequate dose of hyperimmune serum.

While this work was in progress we decided to attempt to produce in adult horses the passive immunity that had been found to exist in the foals from immune dams.

<p align="center">TABLE 6.</p>

<p align="center">*Antibody Titre of Passively Immunized Adult Horses.*</p>

Serum.			Titre of Serum against Strains.		
Number.	Date.	Remarks.	Vryheid.	449.	O.
22000....	24. 8.39	Blood donor; 83 days after immunization.......................	$^1/_{256}$	$^1/_{128}$	$^1/_{128}$
22052.... (control)	24. 8.39	Control not transfused............	—	—	—
	9.11.39	77 Days after receiving strain Vryheid........................	$^1/_{64}$	$>^1/_2$	$>^1/_2$
	23. 2.40	183 Days after strain Vryheid; received vaccine..............	$^1/_{128}$	$^1/_2$	$>^1/_2$
	6. 5.40	73 Days after vaccine...........	$^1/_{128}$	$^1/_4$	$^1/_4$
	24. 6.40	122 Days after vaccine..........	$^1/_{256}$	$^1/_{16}$	$^1/_4$
22053....	24. 8.39	Before transfusion of $2\frac12$ litres of blood.......................	0	0	0
	24. 8.39	Two hours after transfusion......	$^1/_4$	$^1/_2$	$^1/_4$
	9.11.39	77 Days after receiving strain Vryheid........................	$>^1/_2$	$>^1/_2$	$^1/_{64}$
	23. 2.40	183 Days after strain Vryheid; received vaccine..............	$>^1/_2$	$>^1/_2$	$^1/_{64}$
	6. 5.40	73 Days after vaccine..........	$^1/_4$	$^1/_{16}$	$^1/_{64}$
	24. 6.40	122 Days after vaccine..........	$^1/_4$	$^1/_8$	$^1/_{64}$
22054....	24. 8.39	Before transfusion of 5 litres of blood	0	0	0
	24. 8.39	Two hours after transfusion......	$^1/_{16}$	$^1/_8$	$^1/_{16}$
	9.11.39	77 Days after receiving strain Vryheid........................	$>^1/_2$	$>^1/_2$	$^1/_{256}$
	23. 2.40	183 Days after Vryheid; received vaccine.......................	$^1/_2$	$>^1/_2$	$^1/_{256}$
	6. 5.40	73 Days after vaccine...........	$^1/_2$	$^1/_8$	$^1/_{256}$
	24. 6.40	122 Days after vaccine..........	$^1/_2$	$^1/_8$	$^1/_{128}$

NOTE.—On 31.7.40 when the results of the above titrations were known, horses 22052 and 22054 were given an *in vivo* immunity test of pantropic O virus generation 202. Horse 22054, which showed the high titre antibodies, did not react, horse 22052, which showed low titre antibodies to neurotropic strain O, reacted severely after a somewhat prolonged incubation period of 5 days, but recovered. Horse 22053 has been retained for a continuation of this work on a larger scale.

Three susceptible horses (22052, 22053 and 22054) were selected after preliminary tests had shown that their sera contained no demonstrable antibodies. In addition, there was available a horse, 22000, which had received polyvalent vaccine batch 378, 83 days previously in the course of routine tests on the safety and efficacy of

the vaccine issued. On 24.8.39 blood was drawn from the 4 horses to collect serum for neutralization tests. Then blood was transfused for 5 minutes from immune horse 22000 into 22053 and for 10 minutes into 22054; horse 22052 was retained as a control. A very extensive experience in the past has shown that by the technique of jugular transfusion employed (with the length of rubber tubing, bore of canulas and size of attachments used) $2\frac{1}{2}$ litres of blood flows from the donor to the receiver in 5 minutes, and 5 litres in 10 minutes. Two hours after the transfusion further samples of blood were drawn from the receivers and all 3 horses were given a subcutaneous injection of mouse brain emulsion, which by titration in mice was known to contain 100 M.I.D.'s of neurotropic virus, strain Vryheid.

The only adverse effect of the transfusion was that 9 days later horse 22053 developed acute Biliary Fever (*Nuttallia equi* infection) which was controlled by the intramuscular injection of acaprin.

Intracerebral protection tests in mice against strains Vryheid, 449 and 0 were carried out with the serum samples mentioned above and with subsequent samples as detailed in Table 6 (opposite page).

Results: In the first place it is seen that the blood donor showed, in the peripheral circulation, antibodies to high titre against the three virus strains used in the experiment. Blood transfusion resulted in the production of a passive immunity in the receivers as indicated by the presence of antibodies two hours later. Roughly the degree of passive immunity was proportional to the volume of blood transfused; the titre of the antibodies in horse 22054 should have been double that in horse 22053, but actually the difference was fourfold, a discrepancy which is not significant. The low titre of the serum of the passively immunized animals should be compared with the high titres encountered in newly born foals a few hours after sucking.

From this point it is necessary to consider the results obtained with strains Vryheid and 449 as one group; the results with strain 0 (the last column of Table 6) will be examined separately.

The injection of 100 M.I.D. of neurotropic virus at a stage when low titre circulating antibodies were detectable did not result in the production of an active immunity. This conclusion is based upon the finding that the antibody content of the serum of the two horses did not rise, whereas after an interval of 77 days the serum titre of the control had risen to 1/64 and at 183 days stood at double that figure. This antibody production was strain specific since, in the control, neutralizing antibodies to strain Vryheid alone appeared. At this stage all the horses received routine polyvalent vaccine. There was a slight response in each case but the low titre antibodies produced is, in our opinion, related to the presence of heterologous antibodies and is not associated with the development of a solid active immunity against strains Vryheid and 449. Undue significance must not be attached to this result because no untreated susceptible control was available for a test of the vaccine used, but again in our opinion it is highly improbable that the vaccine did not contain more than 100 M.I.D. of strains Vryheid and 449.

The only definite conclusion that can be drawn from this portion of the experiment is that when neutralizing antibodies can be detected in the peripheral circulation the injection of 100 M.I.D. of neurotropic virus will not produce an active immunity. Tentative conclusions drawn are firstly that passive immunity to neurotropic virus may persist for 6 months after the transfusion of $2\frac{1}{2}$ litres of immune blood at which time antibodies are no longer detectable by the technique used; secondly that the presence of antibodies may interfere with active immunization against antigenically different strains, the differences being determined by the mouse protection test.

The results obtained with the neutralization tests against strain O are somewhat bewildering. In the control horse strain Vryheid maintained its antigenic specificity and did not stimulate the production of O antibodies. The subsequent injection of vaccine which contained strain O similarly failed to prove antigenic although as pointed out above, the direct control of this factor was omitted. That the low titre antibodies were not associated with a solid active immunity was supported by the result of the immunity test; the horse reacted severely but recovered. In the other two horses antibodies could be detected in the bloodstream two hours after transfusion. Subsequently there was a steep rise in antibody content which could not have been associated with the injection of the specifically distinct strain Vryheid since it caused no response in the control. The antibody titre was not affected by the injection of vaccine and the correlation between relatively high titre antibody and solid immunity was confirmed by the direct immunity test; the one horse 22054 showed no reaction whatever to the injection of fully virulent pantropic virus. It must be emphasized that there is no error in the antibody titres of the sera as shown by the results of the neutralization tests; naturally this point was subjected to the most critical investigation. The stimulus which produced this solid immunity therefore must remain a matter of conjecture pending further investigation on a larger scale. Accidental natural infection could be ruled out so the most logical assumption is that neurotropic virus persisted in the circulation of the blood donor for 83 days after the immunizing injection and was transmitted at transfusion even in the presence of high titre neutralizing antibodies.

CONCLUSION.

The serum of foals from dams immune to horsesickness did not contain at birth neutralizing antibodies detectable by the intracerebral protection test in mice. Therefore it is assumed that such foals are susceptible to horsesickness although there is at present no direct experimental evidence to support this reasonable assumption. However, it will be appreciated that it is not easy to obtain suitable experimental animals in adequate numbers to be sacrificed in such an investigation.

Within a few hours of sucking the foals developed a high degree of immunity as indicated by the presence of neutralizing antibodies to a high titre in the serum. This solid immunity was confirmed by the results of direct immunity tests on foals that were 40, 82 and

112 days old. The antibody titre of the foal sera was proportional to that of the dam and, after the foals had sucked, reached a value not lower than that of the dam and in 3 out of 5 cases a higher value; moreover the antigenic specificity of the virus strains was reflected in the acquired immunity since the foals showed the presence of immune bodies against only those strains to which the dams were immune. With age there was a gradual decrease in antibody concentration in the foals, the time of detectable persistence being roughly proportional to the initial titre. Roughly speaking this period was about 6 months.

The injection of either virulent pantropic virus or attenuated neurotropic virus at a time when antibodies were present, did not convert the waning passive immunity into a durable active immunity accompanied by persistent high-titre antibodies. The results of injections at a time when the acquired antibodies could no longer be detected by the *in vitro* technique differed. In the case of one foal (22167), the pantropic virus produced a somewhat modified reaction given when the foal was 239 days old even though the foal had survived an injection of the same virus 157 days previously. Using attenuated vaccine virus there was no immunological response even when the foal was 264 days old. It is readily admitted that the significance of this finding must be discounted by the fact that no foals from susceptible dams could be included in the experiments to serve as controls for the response of young animals to vaccine strains of virus. This omission will be rectified after the next breeding season but we know of no valid reason why fully susceptible foals should be refractory to immunization.

The results obtained with the foals were confirmed by analogous experiments on a limited group of passively immunized adult animals. The transfusion of whole blood from an immune donor produced a condition of passive immunity indicated by the results of serum virus neutralization tests. The degree of passive immunity conferred two hours after transfusion was sufficient to control the attenuated virus injected and consequently no active immunity was produced. Even 183 days later there was no response to the injection of routine vaccine. A claim that passive resistance had persisted for this period of six months is not advanced particularly in view of the anomalous finding that a solid immunity to a heterologous strain of virus was found to exist 77 days after transfusion. An attempt is being made to repeat, and if possible, to elucidate this phenomenon.

No direct evidence was collected on the source of the antibodies demonstrated in the foals. In agreement with the conception of Schneider and Szathmary (1939) there was no diaplacental transfer of antibodies. The sudden rise in titre, followed by a gradual fall, indicates either that the antibodies are confined to the colostral milk or if they continue to be secreted in the milk, are not absorbed as unchanged antibodies. At least it is certain that the concentration in the colostrum must be extraordinarily high to produce the high-titre passive immunity that was observed. This view is supported by the work of Mason, Dalling, and Gordon (1930), in their work on the transmission of maternal immunity in the case of the anti-toxin of *Clostridium welchii*, Type B.

DISCUSSION.

No originality is claimed for the observation that foals from immune dams acquire a passive immunity after the first suckle. Since Ehrlichs pioneer work in 1892 the problem of " inherited " immunity has received the attention of a host of workers. No good purpose would be served by reviewing the extensive literature since only a small portion of the published work has been done on the viruses. Schneider and Szathmary (1938-40) in their comprehensive series of articles refer to many of the authors and discuss the findings in details but omit to cite references. Incidentally, these authors clearly imply that the validity of the conclusions drawn by many of the previous workers is open to question because of the failure to draw a distinction between inherited immunity, i.e. diaplacental immunity and acquired immunity, i.e., colostral immunity. In the present work it is claimed that the problem of acquired immunity of foals to a virus disease has been approached on a quantitative basis. Had this been possible say in the case of swine fever, where a great deal of work has been done on the immunization of young pigs, some of the conflicting results which have been reported might have been avoided. For instance, as early as 1910 Reynolds reported that young pigs from immune sows possessed a high grade of immunity, that this temporary immunity lasted about five weeks and could be converted into an active immunity by the administration of a small dose of virus. Benner (1928 and 1930) after concluding that the temporary immunity is acquired either *in utero* or by the colostrum or by both but not by milk, stated that the use of virus alone is too dangerous as a practical method of immunization. Pickens, Reed, Welsh, and Poelma (1928) showed inter alia that young pigs do not always develop an active immunity following the injection of virus, while Burt (1934) stated that it was possible to produce a permanent immunity by the inoculation of 1·5 to 2·0 c.c. of active virus into the offspring of young sows before the age of 18 days. This work on swine fever is analogous to the present work on horsesickness because both the pig and the horse belong to Schneider and Szathmary's group 1, viz., adeciduata, with placenta epitheliochorialis characterized by colostral immunity and absence of diaplacental immunity. Moreover, most of the conflicting points raised by the American workers have been touched on and, because of the quantitative study, have been elucidated. For instance, it has been shown that if virulent horsesickness virus is injected early, the reaction is completely blocked, no durable active immunity is produced and the passive immunity continues to decline at the normal rate; if injected too late the antibody concentration may be too low to control the reaction which then resembles that following the use of an inadequate dose of serum in the simultaneous serum-virus method of immunization. Consequently the degree of passive immunity of each young animal would have to be controlled so carefully that the application of the method to general use would be quite impracticable.

This passive immunity of the new-born foal has a direct bearing on the whole problem of horsesickness prophylaxis in South Africa. Under our conditions of breeding the majority of foals are born from the middle of September to the middle of December. Natural

horsesickness annually makes its appearance about the end of January and increases in severity until the first frosts, say in May. The foals cannot be immunized and yet they are exposed to the greatest danger of natural infection at a time when the acquired passive immunity has declined to a point below the limit of safety. This explains the observed heavy mortality amongst the older immunized foals which prompted this study and indicates that the protection of foals, for the present at least, must remain a problem of management and husbandry and must not be regarded as one of prophylactic immunization.

The work has brought out two additional points which merit further careful investigation; firstly, the apparent interference by one strain of virus with the effect of antigenically heterologous strains injected subsequently, and secondly, the solid immunity produced by transfusion from an immunized animal at a time when the presence of circulating virus would not be expected.

SUMMARY.

1. At birth the serum of foals from immune dams contain no antibodies to neurotropic attenuated horsesickness virus demonstrable by the intracerebral protection test in mice.

2. Less than 30 hours after birth, that is after the first suckle, antibodies are present in a concentration not lower than that of the dam.

3. The titre of the antibodies gradually declines, the duration of their presence being proportional to the original titre. Usually they persist for approximately 6 months.

4. All the evidence indicates that the source of the antibodies is the colostral milk.

5. The acquired immunity was found to protect against virulent pantropic virus for 157 days but not for 239 days. Completely blocked out reactions did not produce a durable active immunity.

6. During the period antibodies could be demonstrated and for an unkown period after their apparent disappearance the inoculation of routine horsesickness vaccine had no immunizing effect.

7. The results obtained in foals were confirmed by passive immunization of adult horses.

8. The significance of the results and their bearing upon routine immunization are discussed.

9. Attention is directed to the necessity for investigating:—

(a) Mutual interference by antigenically different strains of attenuated horsesickness virus.

(b) An observed phenomenon whereby active immunity followed transfusion of blood from an animal showing the presence of high titre antibodies in the serum.

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