PASSIVELY AND ACTIVELY ACQUIRED ANTIBODIES FOR *TRICHOMONAS FOETUS* IN VERY YOUNG CALVES

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(With 2 Figures in the Text)

It is well known that calves are born without any circulating antibody derived from maternal sources *in utero* (Howe, 1921). The plasma of the newly born, fasting animal is devoid of the slow moving electrophoretic components which are identified as serum globulins (Pedersen, 1945). When maternal colostrum is fed to the calf during the first 24–36 hr. of life, the serum proteins it contains pass practically unaltered into the circulation.

Comline, Roberts & Titchen (1951*a*) have shown that after absorption from the small intestine the globulins are carried in the lymph to the peripheral blood via the thoracic duct. They do not enter the portal circulation in any appreciable amounts. The proteins from the colostrum are taken up by the cells of the epithelium of the small intestine and pass directly into the lymph spaces (Comline, Roberts & Titchen, 1951*b*).

The antibodies acquired passively in this way can be detected in the blood of the calf as early as a few hours after the first feed (Kerr & Robertson, 1946).

The present study deals with the disappearance of the passive *Trichomonas foetus* antibody and *Brucella abortus* antibody and with the normal agglutinins for *Trichomonas* which pass into the serum of the colostrum fed calf. The development of normal autogenous agglutinins in the calf and the antibody actively induced by the injection of *T. foetus* antigen into very young animals are also dealt with. Four examples of 'immunological paralysis' which were encountered during the injection of *T. foetus* antigen into young calves are described.

SEROLOGICAL VARIETIES OF *TRICHOMONAS FOETUS*

There are two serological varieties of *T. foetus*, strain (Belfast) B and strain (Manley) M (Kerr & Robertson, 1945). The two types produce clinically identical diseases. They can, however, be distinguished by agglutination with homologous antisera, though the sera of hyperimmunized animals contain a common agglutinin. In low titre antisera, the common agglutinin is not present in sufficient amount to interfere with the distinction between normal agglutinins, detectable with the heterologous strain, and induced antibody, detectable with the homologous strain.

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NORMAL AGGLUTININ (N.A.)

The normal sera of all normal cattle and of horses, sheep, goats, rabbits and man contain agglutinins for *T. foetus*. We have called this naturally occurring agglutinin the non-specific agglutinin in earlier work. We now think it better to call it the normal agglutinin (n.a.) for *T. foetus* because it is absorbed by *T. foetus* but not, for example, by a remotely related flagellate like *Strigomonas oncopelti* nor by the more closely related *T. vaginalis*. Moreover, absorption of normal serum by one serological type of *T. foetus* does not completely absorb the n.a. for the other variety (A. E. Pierce, personal communication). The two serological varieties agglutinate to about the same titre with the n.a., but the strain M being less motile in the conditions of the test tends to give slightly lower readings. N.a. is absent from the serum of the newly born fasting calf, and appears along with colostrum proteins and induced antibodies when the calf has fed on colostrum. Neither the passively acquired n.a. nor the autogenous n.a., when it appears sensitizes the skin (Kerr & Robertson, 1946). The induced antibody to *T. foetus*, on the other hand, whether produced actively or administered passively, may sensitize the animal and the acquisition of skin sensitivity can be followed by the intradermal injection of the test fluid of Feinberg & Morgan (1953).

ANTIBODY AND SERUM GLOBULINS IN THE COLOSTRUM

The globulins in the colostrum are considered by some authorities not to be identical with those in the maternal serum (Burnet & Fenner, 1949). When they have further been passed via the alimentary canal into the serum of the calf they may possibly be again slightly modified. The immunological reactions of the antibody with the antigen, however, are indistinguishable in the maternal serum, the colostrum whey and the serum of the calf. In this paper the use of the words 'antibody and serum globulins' in these different fluids are used without any assumptions about their complete identity.

NOMENCLATURE

As the normal agglutinin has so many characters of an antibody we have used the term 'induced antibody' to describe the agglutinin produced by the injection of *T. foetus* antigen.

The new n.a. arising in the calf itself is called the 'autogenous n.a.' to distinguish it from passive n.a. derived from the colostrum.

METHODS AND MATERIALS

Induced antibody was produced by the intramuscular injection of freeze-dried or acetone-dried whole bodies of *T. foetus*. A few animals were immunized by subcutaneous injections of killed *Brucella abortus* S19 strain. Agglutinin titres for *T. foetus* were determined by the method of Kerr & Robertson using suspensions of the live organisms (Kerr & Robertson, 1941; Pierce, 1947) and sera heated to 56° for 20–25 min.

Digest broth saline (1:3) was used instead of saline for the dilution of the sera and for making the *Trichomonas* suspensions.

Br. abortus titres were determined with formalized suspensions of organisms in the usual way.

RESULTS

The calves were divided into five groups:

Group 1. Colostrum-fed calves of normal mothers (H7, W2, W3).

Group 2. Calves given colostrum containing induced antibodies to *T. foetus* or *Brucella abortus* or both (D13c52, E8c, E7c and P6c52).

Group 3. Calves like those of group 1, actively immunized with *T. foetus* antigen (H5, H6, H8, H9, P8c, P10c, H7 from 96 days onwards and K7c from 171 days).

Group 4. Calves like those of group 2, with passive *T. foetus* induced antibody in their blood, which were then actively immunized with *T. foetus* antigen (K4c and K2c).

Group 5. Calves deprived of the maternal colostrum and treated in various ways (K7c, K10c and P5c52).

It will be convenient to deal first with the decrease of the passive n.a. acquired from the colostrum and the appearance of autogenous n.a. (groups 1-4).

Decreasing passive n.a. titres for Trichomonas foetus and increasing autogenous n.a. agglutinins in colostrum-fed calves

The passive n.a. is present in the serum of all colostrum-fed calves, irrespective of what induced antibodies are also acquired from the maternal colostrum. The initial titres varied considerably and depended partly on the titre of the colostrum, but also upon how much colostrum the calf had taken during the first 24 hr. of life. This applies also to all passively acquired antibodies.

The n.a. of cattle serum has a titre of 1:48 to 1:96. In the first 7 days of life of the calves of normal mothers it varied from 1:6 to 1:96. The time taken for the passive n.a. to decrease to its lowest titre varied in the animals observed, but bore some relation to the early titre (Table 1a). The lowest titres ranged from 0 to 1:6 and occurred from the 23rd to the 45th day. W2, for example, with the high initial

Table 1. *Normal agglutinin titres for Trichomonas foetus in young calves fed on colostrum of normal mothers*

Animals	Disappearance of passive n.a.		Appearance of autogenous n.a.	
	Initial passive titre 1st to 5th day	Day or period of lowest passive titre	Day of rise of titre	Approximate day of full n.a. titre 1:48
(a) Animals received no <i>T. foetus</i> antigen				
W2	1:96	45	52	112
W3	1:6	25-44	51-57	113
H7	1:24	23-50	57	63
(b) Animals received <i>T. foetus</i> antigen				
H6	Missing	55	65	106
H8	1:48	43-50	58	86
H9	1:48	37	60	109
P8c	1:24	17	42	100
P10c	1:48	20	38	74

titre of 1:96 showed only a trace on the 45th day, and W3 with the low initial titre of 1:6 showed no agglutinin from the 25th to the 44th day; but the autogenous n.a. titre began to appear at the same time in the two animals. A stationary low n.a. titre was observed on several occasions.

Five colostrum-fed animals were given intramuscular injections of *T. foetus* antigen at a very early age (Table 1*b*). The active response (see below) did not affect the n.a. titres at this stage. The lowest titres occurred from the 17th to the 55th day.

Table 2. *Normal agglutinin titres for Trichomonas foetus in calves fed on colostrum of actively immunized mothers*

Animal	Immune antibody in colostrum	Disappearance of passive n.a.		Appearance of autogenous n.a. titre		
		Passive n.a. titre 1st to 6th day	Day of lowest passive n.a. titre	Day of rise of n.a. titre	Approx. day of full new n.a. titre	Antigen given
P 13c	<i>T. foetus</i>	1:48	37	44	78	None
E 8c	<i>Br. abortus</i> , <i>T. foetus</i>	1:72	35	n.t.	111	None
P 6c 52	<i>T. foetus</i>	1:48	21	35	92	None
K 2c	<i>T. foetus</i>	n.t.	35	59	90	<i>T. foetus</i> from 35th day
K 4c	<i>T. foetus</i>	n.t.	43	52	n.t.	<i>T. foetus</i> from 29th day

n.t. = no test

In animals with passive, induced antibody derived from the maternal colostrum (Table 2) the lowest passive titre occurred between the 21st and the 43rd day. In these two sets of animals (Tables 1 and 2) the autogenous n.a. appeared about the 7th week of life (42nd to the 56th day) with a variation of about a week either way. The period after which the full n.a. titre was established varied between the 63rd and the 113th day.

The autogenous n.a. did not seem to be related to the presence of passive, induced antibody nor was it influenced by the injection of *T. foetus* antigen. It should be noted that the times of the disappearance and the appearance of the n.a. titres were only approximate, as the tests were usually made at weekly intervals.

Two other calves (group 5) K 10c and P 5c 52, deprived of the colostrum are of interest. In K 10c no passive n.a. could be detected until the 16th day when a trace only was found. The autogenous n.a. appeared on the 24th day when the titre was 1:12.

P 5c 52, had no n.a. up to and including the 22nd day of life and the autogenous agglutinin appeared on the 29th day. The autogenous n.a. titre was 1:48 in these two calves on the 51st and 64th day respectively.

The declining passive titre of induced antibody in colostrum-fed calves and the rising induced antibody in calves receiving injection of Trichomonas antigen

It is proposed to deal first with the disappearing passive induced antibody titres (group 2); then with the rising titres induced by the injection of antigen (group 3); and finally with the rising active titres produced in animals with induced antibody of maternal origin (group 4).

There is a good deal of irregularity in the passive titres in the first days of life. In some cases there was a rapid drop in the first 1–3 days. In others the titre remained unchanged up to the 7th or 8th day. The 'initial' titre was therefore that determined on the 2nd to the 8th day.

Decline in the titre of antibodies to *T. foetus* and *Br. abortus* passively acquired from the colostrum was logarithmic (Table 3). In E7c and E8c the half-life of the passive *Br. abortus* titre was 14–15 days. E8c had both passive *T. foetus* and *Br. abortus* antibodies, and both were eliminated at about the same rate (Fig. 1).

Table 3. *Disappearance in calves of passive induced titres acquired from maternal colostrum*

Animal	Antibody measured	Titre at 1–8 days	Percentage of starting titre found at varying periods (days)		Approximate half life of titre in days
			Day	%	
E7c	<i>Br. abortus</i>	1:640	21	50	14
			35	25	
			52	12·5	
E8c	<i>Br. abortus</i>	1:320	24	50	15
			35	25	
			69	6·25	
E8c	<i>T. foetus</i>	1:192	17	50	14
			35	25	
			47	12·5	
D13c52	<i>T. foetus</i>	1:384	23	50	20
			51	25	
			78	12·5	
P6c52	<i>T. foetus</i>	1:576	35	66	57
			58	50	
			92	33	
			115	25	

In D13c the half-life was about 20 days for the passive, induced antibody to *T. foetus*. The passive n.a. titre (Table 2) declined from 1:48 on the 2nd day to 1:6 on the 37th day (12·5 %). As Fig. 2 shows, the decline in both cases was logarithmic but the slope was different; the passive *T. foetus* antibody reaching 12·5 % of the initial titre in about 75 days and the passive n.a. reaching it in 35 days.

The half-life of the passive *T. foetus* titre in P6c52 was 57 days. This calf ingested 25 lb. of colostrum in the first 24 hr. of life and the elimination of the antibody was very slow.

Two other animals should be mentioned. K4c had a passive titre from the colostrum of 1:192 for *T. foetus* on the 2nd day of life which decreased continuously to 12·5 % at 57 days, giving a half-life of about 17 days. The continuous decrease in the titre was not affected by two injections of antigen of the same serological type on the 29th and 50th day. The second animal, K10c, deprived of colostrum, was given by mouth, in the first 12 hr. of life, a globulin fraction of the colostrum of a cow immunized with *T. foetus*. The initial titre in the blood was 1:36 for *T. foetus* and the half-life 14 days.

Fig. 1. Decline in titre of passive *Br. abortus* agglutinin of maternal origin, $\odot$ — $\odot$; decline in titre of passive specific *T. foetus* agglutinin of maternal origin, $\square$ — $\square$.

Fig. 2. Decline in passive specific agglutinin for *T. foetus* of maternal origin, $\odot$ — $\odot$; decline in passive normal agglutinin for *T. foetus* of maternal origin, $\square$ — $\square$.

*Response to the injection of Trichomonas foetus antigen
in normal colostrum-fed calves*

There were nine calves in this series (K 7c, H 7c, H 6, H 5, E 7c, H 8, H 9, P 8c and P 10c).

Eight received the colostrum of their normal non-immunized mothers; K 7c was deprived of colostrum but did not have antigen injected until the autogenous n.a. titre was fully developed. None of the animals had any passive, induced antibody for *Trichomonas*. E 7c had a passive *Br. abortus* titre and is included in the series because the passive antibody did not interfere with the response to *T. foetus* antigen.

The calves K 7c and H 7c had an autogenous n.a. titre of 1:48 before the first injection. K 7c at 5 months old responded to three intramuscular injections of *T. foetus* antigen as a normal adult would. The skin became sensitized. H 7c at 3 months old responded moderately and the animal was sensitized (Table 4).

Table 4. *Rise of active Trichomonas foetus titre after injection of antigen into calves. One of the animals had passive Brucella abortus titres derived from maternal colostrum*

Animal	State of serum at titre of first injection	Age of calf in days and dose in g. of 1st injections		Immunological response*
		Days	g.	
K 7c	Autogenous n.a. titre 1:48	171	0.2	Good to each dose. Titre 1:768 after 3rd dose
		178	0.3	
		185	0.5	
H 7	Autogenous n.a. titre 1:48	96	0.2	Moderate to each dose. Titre 1:288 after 3rd dose.
		104	0.3	
		110	0.5	
H 6	Passive n.a. titre from normal colostrum 1:18	23	0.3	None to 1st injection. Low titre 1:48 after 2nd dose on 56th day
		56	0.3	
H 5	Passive n.a. titre from normal colo- strum 1:24	13	0.3	None to 1st injection. Low titre 1:48 after 3rd dose on 45th day
		19	0.3	
		45	0.3	
E 7c	Passive n.a. titre 1:24 passive <i>Br. abortus</i> titre 1:640 from colostrum	8	0.1	None to 1st three doses. Low titre 1:48 after 4th dose on 30th day
		15	0.2	
		22	0.4	
		30	0.5	

* In all five animals, the response to all later injections of *T. foetus* antigen was good.

The three animals H 6, H 5 and E 7c were given antigen at an earlier age. Doses given up to and including the 22nd day of life produced no detectable serological response nor had the feeble response to later doses on the 56th, 45th and 30th day respectively any of the qualities of a secondary response. It was, nevertheless, an active response and the animals were sensitized. All these five animals responded well to still later injections of antigen.

The results with four very young calves (H8, H9, P8c and P10c, Table 5) were unexpected. The animals appeared to have their subsequent capacity to respond to the antigen impaired by the early injections. A relatively large amount of antigen was given before the 22nd day during the unresponsive period, with a view to finding whether this massive dosage would promote later production of antibody or in any way affect it.

Table 5. *Immunological reaction of young colostrum-fed calves receiving large doses of Trichomonas foetus antigen in the first 3 weeks of life*

Animal	Age	1st series of injections		Later response
		Dose, antigen (g.)	Response	
H8	8	0.2	0	No response to four injections from 43rd to 79th day and to two on 193rd day and 200th day. Greatly impaired response to injection at 379-390 days
	15	0.3		
	22	0.5		
H9	3	0.2	0	No response to two injections at 81 and 88 days and greatly impaired response to injections at 226-227 days
	7	0.3		
	9	0.4		
	14	0.5		
	18	0.6		
P8c	7	0.2	0	Impaired response to two sets of injections at 121 and 134 days and at 214-225 days
	10	0.3		
	14	0.4		
	17	0.5		
	21	0.6		
P10c	7	0.2	0	Impaired response to two sets of injections at 74-94 days and at 174-185 days
	10	0.3		
	16	0.4		
	20	0.5		
	23	0.6		

There was, as expected, no response to doses given up to and including the 23rd day of life. In H8 the injections were continued from the 43rd to the 79th day and, after a pause of 114 days, from the 193rd to the 200th day without any immunological response. The animal was then rested for 179 days and, when more than a year old (379 days) was given a course of intramuscular injections of 2 g. divided into four doses extending over 11 days. After this treatment the titre rose to 1:192, which is a very poor response for an animal of this age. The n.a. titre at this age is, as a rule, between 1:48 and 1:96. Although the skin did not become sensitized we consider that the 1:192 was due to induced antibody because 26 days after the end of the course of injections the titre fell to 1:72. The results in H9 were very similar to those in H8, and it did not become sensitized. Nevertheless, H8 and H9 reacted normally to injections of killed *Br. abortus* vaccine begun in H8 on the 249th day and on the 131st day in H9, the titre produced being 1:1280 after the third dose.

The responses in P8c and P10c were much the same as those in H8 and H9; they also did not become sensitized. P8c, after three injections totalling 1.3 g. given

between the 121st and 134th day showed a faint rise in titre from 1:48 to 1:96, but the significance of such a rise is doubtful.

In the animals given large doses of antigen in the first 3 weeks of life the highest titre in response to later injections of antigen was 1:192. This is so small a rise upon 1:96 (the common titre of n.a. at that age) that we conclude that the power to respond to the antigen was seriously impaired. There was no sign of rapid secondary responses when the injections were resumed after the long intervals of rest.

The very feeble response should be compared with that of H7c first inoculated when 96 days old (Table 4). This animal responded unmistakably to the first series of injections, and 122 days later reacted rapidly with a rise from 1:96 to 1:768, 7 days after a 0.2 g. dose; and to 1:1536, 20 days after a second dose of 0.5 g. After a further interval of 179 days, 2 g. of antigen in four doses (the amount given to the impaired animals) produced a rise to 1:3072. It is proposed to rest these animals for a year and to see if they will then react normally to *T. foetus* antigen.

There remain to be considered two animals, K2c and K4c (group 4) actively immunized with *T. foetus* antigen in the period when *T. foetus* antibodies of maternal origin were declining in the serum. In K2c the initial passive titre of 1:192 (strain B) had decreased to 1:48 at 35 days when the antigen (strain B) was given in three injections totalling 0.6 g. at 3-day intervals from the 35th to the 42nd day. The titre declined to 1:36 on the 38th day, but 6 days after the third dose rose to 1:72; although low, this was at least a measure of actively induced antibody because the n.a. titre was 1:12.

K4c with a passive, induced antibody titre of 1:48 at 29 days, did not respond to 0.3 g. of antigen (strain B) injected on that day, and the passive titre decreased further to 1:24 in spite of a 2nd injection of 0.3 g. at 50 days. A similar dose given at 72 days produced a low titre of 1:72 at 78 days. This was in part at least active agglutinin as the autogenous n.a. titre was 1:24. These two animals responded to small doses of antigen given at 6–10 weeks old in spite of the presence of passive, induced antibody.

DISCUSSION

We consider that the normal agglutinin for *T. foetus* is a native constituent of cattle serum and not induced by an exogenous antigen. It is a globulin, and has the nature of an antibody in that it specifically sensitizes *Trichomonas* to the lytic action of complement. Each of the two serological varieties of *T. foetus* absorbs all the n.a. for itself, but leaves a reduced amount of n.a. for the other variety. The normal agglutinin for *T. foetus* is not absorbed by *T. vaginalis*.

There does not seem to be any means of obtaining rigorous proof of its endogenous origin, but there are a good many facts supporting it. Thus the injection of *T. foetus* antigen has no effect upon the disappearance of the passive n.a. titre derived from the colostrum or on the development of the new normal agglutinin arising in the calf itself.

The remarkably constant titre of the n.a., once it has reached its full development, not only in the one animal, but in cattle in general, would be difficult to explain on any theory of infection either by *T. foetus* or by an organism sharing an

antigen with *T. foetus*. Induced antibody to *T. foetus* fluctuates widely in amount and constantly decreases after immunization. The n.a. differs from the induced antibody in that it does not produce hypersensitivity in the skin. The fact that passive, normal agglutinin decreased independently of the passive, induced agglutinin when both were present in the same animal emphasizes their difference.

The passive, induced antibody against organisms as far apart as *Br. abortus* and *T. foetus* declined at approximately the same rates when both were present in the same animal. The variation in rate of the decrease in *T. foetus* passive (induced) antibody in different individuals seemed to depend in part upon the amount of colostrum taken. The decrease was approximately logarithmic and the half-life of the titre varied from 14 to 20 days. It was 57 days in one healthy calf that took 25 lb. of colostrum.

In calves, Smith & Holm (1948) found a half-life of 50 days for passive *Haemophilus pertussis* antibody from the colostrum. Dixon, Talmage, Maurer & Deichmüller (1952) measured the half-life of homologous γ -globulin passively administered to various animals, and found that it varied very considerably from species to species and among different age groups within a species.

The response of the very young animal to antigen has not been the subject of much detailed study. Barr, Glenney & Howie (1953), using lambs that had had the colostrum of mothers actively immunized with diphtheria toxoid, concluded that young lambs, in most cases, made some response to injected toxoid from the 14th day of life onwards.

With *T. foetus* antigen there was no serological response to very early intramuscular injection. The first unmistakable response occurred after an injection given at 30 days. In four animals, where the amount of antigen injected in the first 3 weeks of life was considerable (1–2 g.) not only was there no immediate response, but there was a subsequent paralysis or impairment of the capacity to react to a second course of the same antigen. The impairment was evident on injecting a third course of antigen after a rest of as much as 179 days.

The system we have to use here is unsuitable for detailed study of immunological paralysis, because the antigen is crude and not very potent, and the presence of normal agglutinin in relatively high titre greatly enhances the difficulty of estimating the significance of very slight rises in titre. This example of immunological paralysis, however, does not stand alone. The polysaccharide antigen of the pneumococcus when injected in sufficiently large doses into mice inhibits the antibody response to a subsequent optimal immunizing dose (Felton, 1949). This was termed 'immunological paralysis' and was type specific. The mice, like the calves described above, responded to other antigens in the normal way. In such mice the polysaccharide persisted for at least 52 weeks.

The exhaustion of various antibody-producing sites, notably the spleen, has been demonstrated by Taliaferro & Taliaferro (1951) in rabbits receiving multiple injections of antigen (sheep red blood cells). The site passes into an inactive state during which little or no antibody is produced until after a period of rest and regeneration.

In a recent study briefly reported by Dixon & Maurer (1953) immunological

unresponsiveness was produced in rabbits by daily injection of large amounts (10 ml. of a 5% solution per kg. body weight) of bovine serum albumin over a period of 6–12 weeks.

SUMMARY

1. The normal agglutinin (n.a.) for *Trichomonas foetus* found in all normal adult cattle has a titre of 1:48 to 1:96. N.a. appears to be specific for *T. foetus* and cannot be absorbed by other flagellates such as *T. vaginalis* or even completely by the heterologous serological variety of *T. foetus* itself. The n.a. appears to be a native constituent of the serum and not to be induced by an exogenous antigen.

2. The n.a. passes into the serum of the calf from the maternal colostrum during the first 24 hr. of life. The elimination of the passive n.a. and the development of the autogenous newly formed n.a. in the calf are traced. The passive n.a. disappeared from the 17th to the 55th day and the autogenous n.a. began to appear as a rule from the 35th to the 60th day and was fully established by the 63rd to the 113th day.

3. The elimination of the maternal, induced antibody acquired passively from the colostrum took place logarithmically. The rate measured by the half-life ranged from 14 to 20 days, but in one animal which took a very large amount of colostrum it was 57 days.

4. The intramuscular injection of *T. foetus* antigen into very young calves up to 4 weeks old induced no antibody. When the doses were relatively small the animal produced antibody to antigen given at a later period. When a very large amount was injected in this early period the subsequent capacity to respond to the same antigen but not to other antigens was seriously impaired.

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REFERENCES

- BARR, M., GLENNY, A. T. & HOWIE, J. W. (1953). *J. Path. Bact.* **65**, 155.
BURNET, F. M. & FENNER, F. (1949). *Production of Antibodies*, 2nd ed. Melbourne: Macmillan and Co.
COMLINE, R. S., ROBERTS, H. E. & TITCHEN, D. A. (1951a). *Nature, Lond.*, **167**, 561.
COMLINE, R. S., ROBERTS, H. E. & TITCHEN, D. A. (1951b). *Nature, Lond.*, **168**, 84.
DIXON, F. J., TALMAGE, D. W., MAURER, P. H. & DEICHMÜLLER, M. (1952). *J. exp. Med.* **96**, 313.
DIXON, F. J. & MAURER, P. H. (1953). (*Abstracts*), 4th Int. Congr. Microbiol., Rome 1953. pp. 320. vol. I.
FEINBERG, J. G. & MORGAN, W. T. J. (1953). *Brit. J. exp. Path.* **34**, 104.
FELTON, L. D. (1949). *J. Immunol.* **61**, 107.
HOWE, P. E. (1921). *J. biol. Chem.* **49**, 115.
KERR, W. R. & ROBERTSON, M. (1941). *Vet. J.* **97**, 351.
KERR, W. R. & ROBERTSON, M. (1945). *Vet. Rec.* **57**, 221.
KERR, W. R. & ROBERTSON, M. (1946). *J. comp. Path.* **54**, 38.
PEDERSEN, K. O. (1945). *Ultracentrifuge Studies on Serum and Serum Fractions*. Upsala.
PIERCE, A. E. (1947). *Lab. J.* **8**, 238.
SMITH, E. L. & HOLM, A. (1948). *J. biol. Chem.* **175**, 349.
TALIAFERRO, W. H. & TALIAFERRO, L. G. (1951). *J. infect. Dis.* **89**, 143.