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The immune response to viruses in calves

II. The response in young calves

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INTRODUCTION

There have been a number of studies carried out to determine the immune response in young animals: for example, using bacteriophage as antigen (Uhr *et al.* 1962; Silverstein *et al.* 1963; Kim, Bradley & Watson, 1966), bacterial antigens (Sterzl, *et al.*, 1964; Bellanti *et al.* 1963) and poliovirus as antigen (Svehag & Mandel, 1964*b*). In general, it has been found that the young animal produces an early IgM response with a relatively delayed IgG, and the responses are of lower magnitude than those of mature animals. Probably the one clear feature to emerge from this work is the variation between different species, and also between different antigens in any one species. Studies on young calves have been restricted to either field trials of vaccines (Henning, 1953) or experiments involving serological tests without antibody characterization (Lambert *et al.* 1961; Smith & Ingram, 1965; Dawson, Darbyshire & Lamont, 1965).

This paper describes experiments on the response to viruses in young calves using techniques of protein separation to study the part played by physically different immunoglobulins.

MATERIALS AND METHODS

The serological tests and techniques of protein separation have been described in the previous paper (Sanderson, 1968*a*).

Viruses

The group B arbovirus Murray Valley encephalitis (MVE) was used as infected unweaned mouse brain homogenate of 10^8 LD 50/0·1 ml. in unweaned mice; the dose was 2·5 ml.

The group A arbovirus Getah (strain N 544) was used as infected unweaned mouse brain homogenate of 10^5 LD 50/0·1 ml. in unweaned mice; the dose was 2·5 ml. Full details of these viruses are given elsewhere (Sanderson, 1968*b*).

Parainfluenza 3 virus (PI 3)—a strain from man (obtained from Commonwealth Serum Laboratories, Parkeville, Victoria)—was propagated in primary bovine kidney cells and was 10^6 TCID 50/0·1 ml.; the dose was 4 ml.

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Reovirus 1 (Reo)—Lang strain (Stanley, 1961)—was propagated in primary bovine kidney cells and was $10^{6.5}$ TCID 50/0·1 ml.; the dose was 4 ml.

Experimental design

The principal of the experiment was to inject virus preparations at different ages over the first weeks of life, to collect bi-weekly serum samples over a period of 4 weeks for antibody assay, and then to give a second injection to test for a secondary response. To increase the amount of data obtained from a small number of animals four viruses were used at different times in each calf. The experiment is set out in Table 1. Eight calves were obtained from local sales. In most cases the ages were obtained from the owner, while the age of some was estimated from the condition of the umbilical cord and the general appearance. Neither of these could be considered completely reliable. A blood sample was taken and the calves given the first injection on the day after arrival. The eight calves were treated as pairs, and each pair was injected subcutaneously with a different virus at intervals of 7 days until after 4 weeks each animal had received an injection of the four viruses. Over the next 4 weeks the same sequence was repeated, so that each animal received a second injection of the same virus 4 weeks after the first. The calves were kept in insect-free housing.

Table 1. *Experimental design; calf identification number and age at each injection*

(Age in days shown in parentheses.)

Calf no.							
99	100	101	102	103	104	105	106
First injection							
MVE (12)	MVE (4)	Reo (8)	Reo (8)	Get (6)	Get (11)	PI3 (11)	PI3 (11)
Reo (19)	Reo (11)	Get (15)	Get (15)	PI3 (13)	PI3 (18)	MVE (18)	MVE (18)
Get (26)	Get (18)	PI3 (22)	PI3 (22)	MVE (20)	MVE (25)	Reo (25)	Reo (25)
PI3 (33)	PI3 (25)	MVE (29)	MVE (29)	Reo (27)	Reo (32)	Get (32)	Get (32)
Second injection							
MVE (40)	MVE (32)	Reo (36)	Reo (36)	Died	Get (39)	PI3 (39)	PI3 (39)
Reo (47)	Reo (39)	Get (43)	Get (43)	—	PI3 (46)	MVE (46)	MVE (46)
Get (54)	Get (46)	PI3 (40)	PI3 (50)	—	MVE (53)	Reo (53)	Reo (53)
PI3 (61)	PI3 (53)	MVE (57)	MVE (57)	—	Reo (60)	Get (60)	Get (60)

This experimental design allowed a greater number of responses to be studied in a small number of calves, but also provided a considerable economy in the separation techniques, as each fraction could be tested with each of the viral antigens.

RESULTS

The expected total number of responses was not realized because all eight calves possessed maternally derived antibody to PI 3. Two calves (99 and 101) possessed maternal antibody to Reo. One calf (103) died without obvious clinical symptoms or gross pathological lesions. This occurred 5 days after an injection of Reo although there was no reason to believe that the two events were related. For these reasons only twenty-one responses were studied, consisting of eight to MVE, eight to Getah and five to Reo. In addition, the levels of maternal antibody to PI 3 and Reo were sufficiently low in one calf to allow a detectable response to the second injection. The presence of maternal antibody allowed a comparison of this immunoglobulin with autogenous antibody by the same techniques of physical separation.

Each serum sample was tested by the haemagglutination-inhibition (HI) test and the haemolytic complement fixation test (HCFT). Selected samples were then tested by HI after zone centrifugation; this technique allowed the detection of lower levels of antibody than the test on whole serum and so the interpretation of the experiment is based on these results. For this reason the results of the HI and HCFT on whole serum are not tabulated. The results are shown in Figs. 1–3.

Table 2. *Summary of the type of response after the first and second injection of virus, showing the types of immunoglobulin synthesized*

(Calf number with age at injection in days in parenthesis.)

	Response to first injection			Response to second injection		
Virus	IgM + IgG	Only IgG	No response	IgM + IgG	IgG	Primary type
MVE	99 (12)	—	—	—	99	—
	—	100 (4)	—	—	100	—
	—	—	101 (29)	—	101	—
	102 (29)	—	—	—	102	—
	103 (20)	—	—	—	Died	—
	—	—	104 (25)	104	—	—
	—	—	105 (18)	105	—	—
	—	106 (18)	—	—	106	—
Getah	—	99 (26)	—	—	99	—
	100 (18)	—	—	—	100	—
	101 (15)	—	—	—	101	—
	102 (15)	—	—	—	102	—
	—	—	103 (6)	—	Died	—
	104 (11)	—	—	—	104	—
	—	105 (32)	—	—	105	—
	—	—	106 (32)	—	—	106
Reo	100 (11)	—	—	—	100	—
	102 (8)	—	—	—	102	—
	104 (32)	—	—	—	104	—
	105 (25)	—	—	105	—	—
	—	—	106 (32)	—	106	—

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Response to the first injection

After the first injection of each virus the calves reacted either by producing IgM followed by IgG, or by producing IgG without detectable IgM. In some no antibody was detected in the 28 days before the second injection (Table 2).

(i) In response to the first injection of MVE (Fig. 1), five of the eight calves formed detectable antibody but only three of these formed detectable IgM

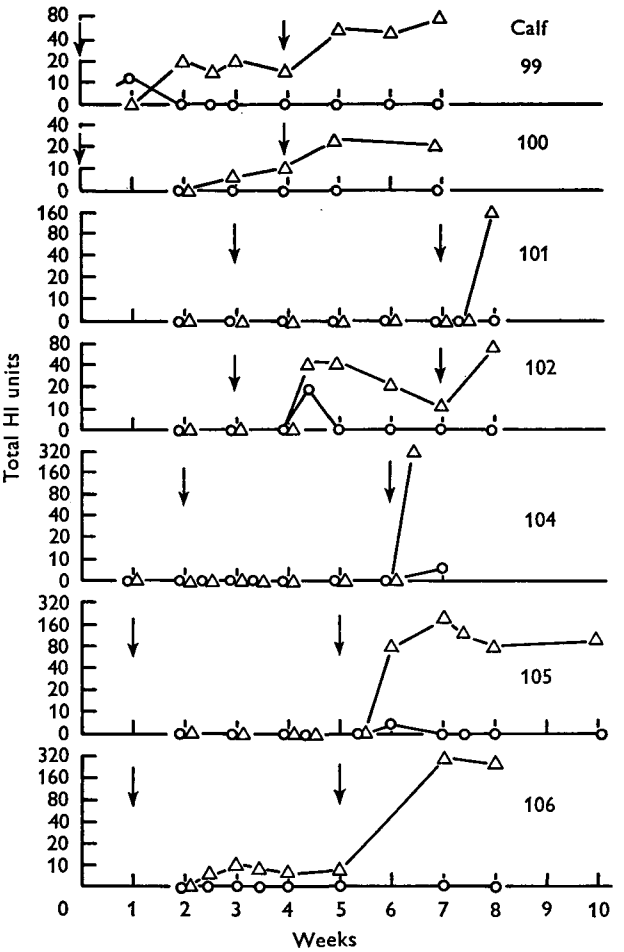

Fig. 1. Response to MVE shown as the total HI units in IgM and IgG fractions of sucrose gradients. Calf 103 is excluded. The arrows indicate the days on which virus was injected. All samples (bi-weekly) were tested for antibody in whole serum, in each case pre-inoculation samples did not contain antibody. △, IgG; ○, IgM.

(Table 2); in two of these the IgM was low in titre and only persisted for a short time. The other animal (103) died before the completion of the experiment and so is not included in Fig. 1. The IgM was first detected by the 7th or the 10th day and IgG appeared by the 10th or 14th day (Table 3).

(ii) In response to Getah (Fig. 2) all but two calves formed detectable antibody

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and four of the six formed IgM (Table 2) which appeared by the 7th day in three and by the 17th in one calf (Table 3). In calf 100 IgM persisted for at least 3 weeks although in the others it was of short duration. The IgG was detected much later than had been the case with MVE, varying from 17 to 28 days after injection. The two calves not responding (103 and 106) are omitted from Fig. 2.

Table 3. *The number of days after each injection before HI antibody was detected, and the duration of IgM*

(Calf number with age at injection in parenthesis. —None detected.)

			Primary response		Secondary response	
Virus	Calf no.	Injection no.	IgM (duration)	IgG	IgM	IgG
MVE	99 (12)	1st	7 (7)	14	—	7
	100 (4)	1st	—	14	—	7
	101 (29)	4th	—	—	—	7
	102 (29)	4th	10 (7)	10	—	7
	103 (20)	3rd	10	10	—	Died
	104 (25)	3rd	—	—	7	7
	105 (18)	2nd	—	—	4	7
	106 (18)	2nd	—	10	—	7
Getah	99 (26)	3rd	—	28	—	7
	100 (18)	3rd	7 (21)	21	—	3
	101 (15)	2nd	7 (7)	17	—	7
	102 (15)	2nd	7 (7)	21	—	7
	103 (6)	1st	—	—	—	Died
	104 (11)	1st	17 (7)	17	—	7
	105 (32)	4th	—	28	—	3
	106 (32)	4th	—	—	—	—
Reo	100 (11)	2nd	7 (21)	14	—	7
	101 (8)	1st	—	—	—	7
	102 (8)	1st	3 (18)	3	—	7
	104 (32)	4th	7 (7)	14	—	7
	105 (25)	3rd	7 (21)	7	7	7
	106 (25)	3rd	—	—	—	7

(iii) In response to Reo (Fig. 3) four of five calves formed IgM and IgG (Table 2). The response in 99 and 101 was complicated by maternal antibody and is not included in this table. The IgM was in general of higher titre than the IgM to the other viruses and appeared by the 7th day in three calves and by the 3rd day in one calf (Table 3). IgG was detected from 3 to 14 days after injection. As the IgG was usually detected well before the end of the 28-day period with all three viruses, it is unlikely that the lack of detectable IgG in the other calves was due to a response delayed beyond the 28 days of the experiment.

All except calf 106 produced IgM in response to at least one virus. This indicated that the ability to respond with IgM was diminished rather than deficient in individual calves. There was no observable relationship between the type of response and the age at injection or the injection sequence. None of the calves with maternally derived antibody formed IgM and there was no increase in IgG levels.

Response to the second injection

After the second injection a secondary response occurred in all but one case (Table 2). This was calf 106, which had not responded to the first injection of Getah and a low titre response of the primary type followed the second injection. The type of immunoglobulin involved in this response was not determined and so it is omitted from Fig. 2. Only three of the secondary responses contained IgM and even in these it represented a very small proportion of the total antibody.

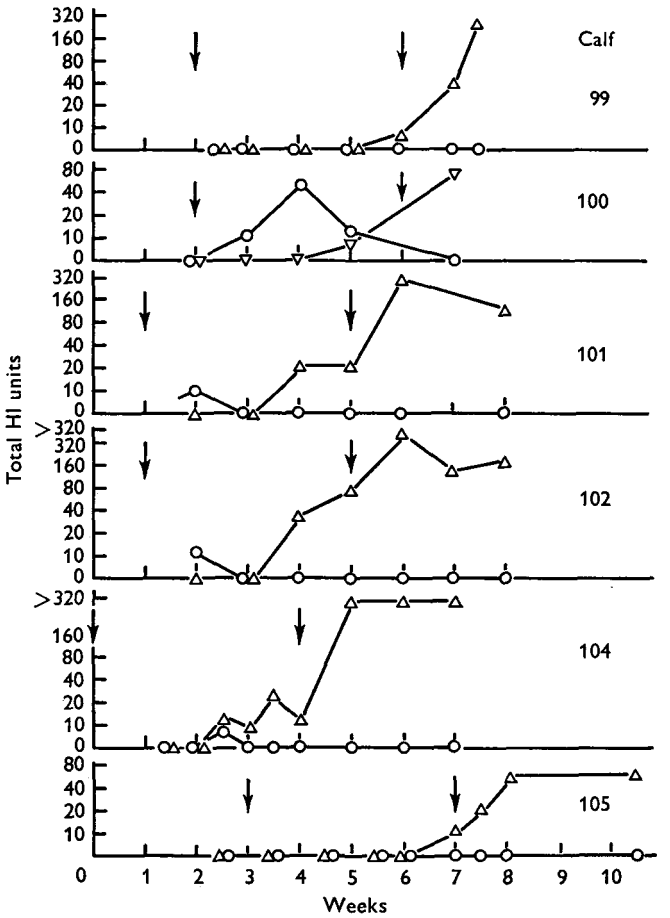

Fig. 2. Response to Getah (as in Fig. 1). Calves 103 and 106 excluded. △, IgG; ○, IgM.

In calf 101 the levels of maternally derived antibody to PI3 and Reo were low. The second injection of Reo gave a marked secondary response (Fig. 3), indicating that the passive antibody was of sufficiently low titre to allow priming for a secondary response. The second injection of PI3 caused a response which seemed most likely to be a primary response in that the antibody titre was of a low level on the 7th day (Fig. 3). There was no IgM detected in this response. In none of the other calves with passive antibody did the second injection result in a detectable increase in antibody.

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To further examine the properties of the IgG at different phases of the response, various samples were examined by anion exchange chromatography and by electrophoresis on acrylamide gel. The serum samples tested were those taken at the following weeks after the beginning of the experiment: calf 99, 4 and 6; calf 100, 3 and 4; calf 101, 1, 4 and 6; calf 102, 1, 2, 5 and 6; calf 104, 5 and 6; calf 105, 3 and 6; calf 106, 3, 5 and 6. These times correspond to the weeks in Figs. 1–3.

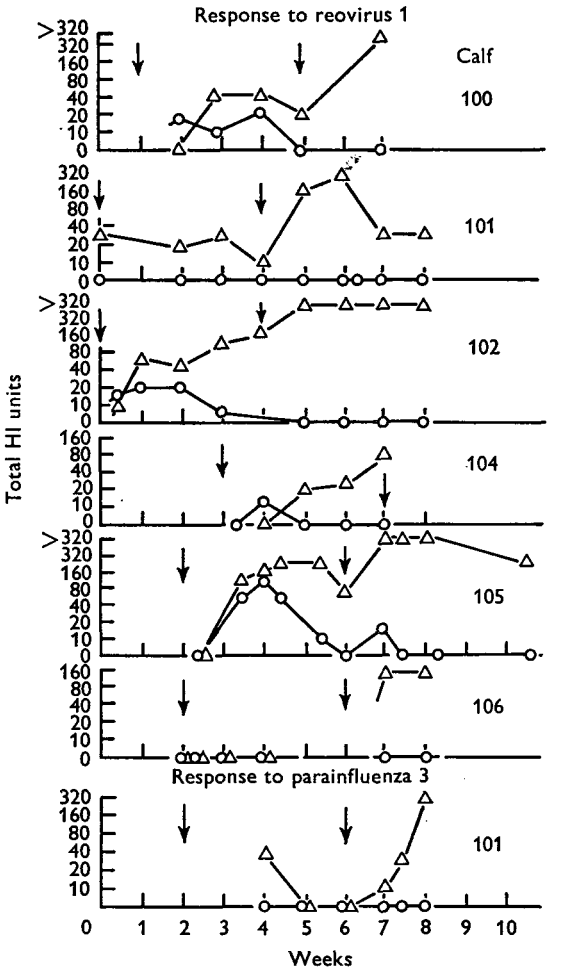

Fig. 3. Response to Reo (as in Fig. 1). Calf 101 was the only animal with pre-inoculation (maternal) antibody. Response to PI3 in calf 101; maternal antibody had disappeared by the fifth week and the animal responded to the second injection of virus. △, IgG; ○, IgM.

In only one sample was antibody detected in the IgG_S fractions (1/2 was the lowest dilution tested). This was in calf 102 during the secondary response to Reo (6th week) when the HI titre was 2560, and the titre in the IgG_S was 32. After separating by electrophoresis, the antibody of both maternal and autogenous origin in all samples was restricted to the IgG_F zone. That maternal antibody is IgG_F has been shown by Murphy *et al.* (1964) and Pierce & Feinstein (1965).

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The IgG_S from calf 102 with an HI titre of 32 had no complement-fixing activity at a dilution of 1/2, suggesting that the differences in chromatographic properties reflected different biological properties.

After the secondary response most of the calves showed a marked drop in antibody titre, which in some cases fell to a very low level within a few months (Table 4).

The properties of antibodies to MVE were studied by the plaque reduction test, which indicated both neutralizing and plaque enhancing activity. These results are reported in detail elsewhere (Sanderson, to be published.)

Table 4. *Duration of antibody after second injection*

			Months after second injection								
Virus	Calf	Test	0	1	2	3	4	5	6	7	8
MVE	101	HI	160	160	40	40	20	—	10	—	—
		HCFT	40	40	10	10	< 10	—	< 10	—	—
		NT	6	—	—	—	0	0	0	—	—
	106	HI	160	80	80	40	10	—	—	—	10
		HCFT	40	40	40	—	< 10	—	—	—	—
		NT	6	6	—	4	3	—	—	—	1
Getah	101	HI	80	—	—	—	40	—	—	—	—
		HCFT	160	—	—	—	20	—	20	—	—
	106	HI	10	10	< 10	—	—	—	—	—	—
		NT	3	4	2	—	0	—	—	—	—
Reo	101	HI	160	10	< 10	—	—	—	—	—	—
		HCFT	40	< 10	—	—	—	—	—	—	—
	106	HI	160	10	< 10	—	—	—	—	—	—
		HCFT	160	—	—	—	—	< 10	—	—	—
PI3	101	HI	40	80	80	20	40	—	10	—	—
		HCFT	20	20	—	10	—	—	10	—	—

DISCUSSION

Thirty-two possible responses were given by the use of four different viruses in eight calves. This number was reduced to twenty-one by the death of one calf and the presence of maternal antibody to PI3 in all eight calves and to Reo in two calves.

Three types of response occurred after the first injection of virus. These consisted of either sequential synthesis of IgM and IgG, IgG with no detectable IgM or no response at all (Table 2). There was no IgM and no increase in IgG titre in the presence of maternal antibody. In one calf there was priming for a secondary response in the presence of low levels of maternal antibody.

The most surprising finding in this experiment was the virtual absence of IgM in the primary response to MVE and Getah in the young calves. Only one calf formed a significant amount of IgM in response to either of these viruses, this was calf 100 (Fig. 2) in response to Getah. In those cases in which IgM was produced it was usually detected by the 7th or 10th day, which was not greatly different from the response in the older calves of the previous paper. The time before IgG was

first detected was in general much longer and more variable than the IgG response in older calves (Table 3). A relatively low level of IgM which was rapidly replaced by IgG at much higher titre was reported in the young rat (Williams, 1966), but in general the young animal is regarded as producing relatively more IgM (Uhr *et al.* 1962; Bellanti *et al.* 1963; Silverstein & Kraner, 1964; Sterzl *et al.* 1964; Buffe & Burtin, 1967).

In all the calf responses where IgM was detected it was followed by IgG, but in four cases IgG was detected without IgM and in most of the other responses the IgM was of a remarkably transient nature. This suggested that in calves at this age the induction requirements for IgG were lower than the requirements for the formation of IgM. The reverse situation seems to be the more general rule in the adult animal (Uhr & Finkelstein, 1963; Svehag & Mandel, 1964*a*, *b*), although Williams (1966) found that rats injected on the day of birth gave a secondary response to a later injection in the absence of a primary response.

In the secondary responses IgM was either not detected or occurred at very low levels, and this was the case in six secondary responses in which there was no detectable antibody in the primary response. This is different from the situation in rabbits, where similar amounts of IgM appear in both the primary and the secondary response (Svehag & Mandel, 1964*b*), and in rats where an increased amount of IgM can be demonstrated in the secondary response (Nossal, Austin & Ada, 1965).

The fact that calves can be immunologically primed without giving a serological response indicates that vaccine trials should be based on the ability to stimulate immunological memory. This state is tested where vaccination is measured by resistance to challenge. These results provide an explanation for the observation by Henning (1953) and Lambert *et al.* (1961) that vaccination of calves produced good field immunity but the serological response was of poor grade.

SUMMARY

Injections of virus in calves up to about 5 weeks of age caused either the production of both IgM and IgG, or the production of only IgG, or no detectable antibody. In all but one case priming for a secondary response occurred even in the absence of a detectable primary response. These results suggest that in the young calf immunological memory is more readily induced than IgG synthesis. IgM appeared by the 7th or 10th day, which was similar to the response in older calves. IgG was more variable in its appearances but was usually considerably delayed relative to the response in older calves. In general only low levels of IgM were formed in the primary response and it was virtually absent from the secondary responses. Higher levels of IgM were formed after reovirus inoculation than after Murray Valley encephalitis virus or Getah viruses. The delay in appearance of IgG was more pronounced in response to Getah than to reovirus or Murray Valley encephalitis virus. High levels of maternally derived passive antibody inhibited the development of an active response, although in one animal a response occurred in the presence of low levels of passive antibody.

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