Arch. Inst. Razi, 1971, 23, 79 - 88

Indirect Complement Fixation in Foot–and–Mouth Disease (*)

II. Screening of Cattle Serums

By

E. Traub, M. Hessami and A. Shafyi

In the preceding communication (6) it was shown that indirect complement fixation (ICF) can be used to study antibody response to foot-and-mouth disease (FMD) antigen in cattle and sheep.

The present report is concerned with application of ICF as a simple and rapid screening procedure to detect susceptible cattle in the field.

Materials and Methods

Bovine serums

Before starting work with ICF, serums from cattle intended for purchase as experimental animals were screened by neutralization tests (NT) in tissue cultures, first in primary cultures of calf kidney cells and later in ovine embryo kidney cells, preferably from fat-tail sheep which are highly susceptible to the virus types under study (A, O and SAT 1). Many of these serums were kept deep-frozen for later work with ICF. A total of 148 bovine serums was tested in both NT and ICF.

Some serum samples originated from cattle purchased by Razi Institute at Teheran slaughterhouse, but most were collected at farms in the Teheran region, where we thought susceptible animals might be found. Serum donors were 6 to 18 months old at the time of bleeding.

Indirect CF and neutralization tests

Procedures used in both tests were as described previously (6). In ICF, serums were tested with BHK antigens of Types A, O and SAT 1, in NT against unmodified virus of Types A and O. Only one test was carried out with SAT 1 virus. The constant serum-virus dilution method was used in NT and neutralization indices (NI) were calculated according to the method of Reed and Muench (4).

(*) Zbl. Vet. Med., B, 15, 433-442, 1968.

Results

Presence of inhibitors in bovine serums

Some normal bovine serums contain non-specific inhibitors which are disturbing factors in both tests, especially ICF. The mode of reaction of sera containing ICF inhibitor is shown in Table 1 (Nos. 4799, 4724, 3782 and 54).

Table 1

Reaction of bovine serums containing inhibitor

Test No.	Cattle No.	i. l. challenge (Type and result)	Serum tested and date of bleeding		NI (log_{10})		Antigen titration †			Serum titration ††		
					A	O	A	O	SAT 1	A	O	SAT 1
1	4799	A + + *	N	19 - 2 - 67	1.0	1.2	17	0	1	80	63	66
			N	26 - 2 - 67	- 0.5		20	0	1	84	62	67
			C	18 - 3 - 67	> 4.7		0	0	1	51	62	70
	4798	A + +	N	19 - 2 - 67	3.0	1.7	98	46	28	100	100	100
			N	26 - 2 - 67	1.5		99	43	31	100	100	100
			C	18 - 3 - 67	> 2.7		2	49	37	70	100	100
	4759 Co.	A + +	N	19 - 2 - 67	0	0.7	96	53	37	100	100	100
			N	26 - 2 - 67	1.2		98	48	25	100	100	100
			C	18 - 3 - 67	> 4.2		0	17	39	54	98	100
2	4724	A + +	N	25 - 3 - 67	- 0.7		18	4	38	92	76	97
			N	23 - 4 - 67	- 1.0	0.2	9	0	8	78	63	89
			C	7 - 5 - 67	> 5.0		0	0	6	35	68	67
	4794 Co.	A + +	N	25 - 3 - 67	0.2		66	74	65	100	100	100
			N	23 - 4 - 67	0.2	0.2	63	76	67	100	100	100
			C	7 - 5 - 67	> 4.7		0	40	69	54	100	100
3	3782	O + +	N	24 - 5 - 67	0.2	1.0	28	24	20	94	94	97
			N	4 - 6 - 67		- 1.0	2	4	1	84	81	81
			C	12 - 6 - 67		4.0	1	1	1	57	16	57
	54	O + +	N	29 - 4 - 67	- 0.2	0	95	77	76	100	100	100
			N	4 - 6 - 67		- 0.7	23	21	9	91	92	89
			C	12 - 6 - 67		3.7	1	0	0	79	67	59
	67	O + +	N	29 - 5 - 67	1.2	2.0	87	59	56	100	100	100
			N	4 - 6 - 67		1.0	79	50	37	100	100	100
			C	12 - 6 - 67		2.0	45	0	18	100	55	100
	56 Co.	O + +	N	29 - 4 - 67	0	0	84	77	78	100	100	100
			N	4 - 6 - 67		- 1.0	75	57	51	100	98	97
			C	12 - 6 - 67		3.7	3	1	12	87	64	91
	negative control serum				- 0.7	0	98	71	70	100	100	100

Abbreviations: i. l. = intralingual
N = normal serum
C = convalescent serum

NI = neutralization index

Co. = control

\+ figures indicate mean fixation (%) by undiluted BS-antigen mixture and dilutions 1 : 1.5 to 1 : 8

++ mean fiaxtion (%) by mixtures of undiluted antigen and BS (undiluted to 1 : 128)

* generalized FMD

Sera carrying much ICF inhibitor strongly agglutinated red blood cells of sheep.

The 4 cattle just mentioned carrying ICF inhibitor of different potency were included as additional controls in intralingual (i. l.) challenge tests to determine whether or not inhibitor made them resistant to FMD. Also included in these tests were 2 cattle (Nos. 4798 and 67) whose normal serums showed suspicious NI, probably attributable to non-specific neutralization inhibitor.

As Table 1 shows, all these animals proved fully susceptible to i. l. infection with either Type A or O. They developed generalized FMD which did not differ in severity from that in the actual controls (Nos. 4759, 4794 and 56). In addition, bovine No. 69 carrying neutralization inhibitor (NI = 2.0 log., see lated i. l.

Table 3) was fully susceptible to a small dose of virus (about 10 ID_{50}) inocu-

NI of serums from animals with ICF inhibitor were within, or slightly beyond, the acceptable range, and serums from cattle with neutralization inhibitor did not inhibit ICF.

It was repeatedly observed that inhibitor for ICF may differ in amount in normal serums obtained from the same bovine on different days. Examples in Table 1 are Nos. 4724, 3782 and 54. No. 54 apparently had no inhibitor on 29-4-67, but was inhibitor-positive on 4-6-67. Infection with FMDV caused an increase in ICF inhibitor in oNs. 4724, 3782 and 54 (see serum titration).

Table 1 further shows that inhibitor for ICF reacted with antigens of all three types.

Among 148 serums tested, 12 (= 8%) contained strong inhibitor for ICF. A number of serums probably contained weak ICF inhibitor, but this did not disturb the tests to a great extent.

Screening tests

For presentation in Tables 2 to 5, batches of bovine serums were selected from

(a) groups of cattle purchased by Razi Institute from Teheran abattoir (Table 2);

(b) herds without evidence of recent infection but presence of serum inhibitors (Table 3);

(c) herds in which specific antibodies and strong inhibitor for ICF were not detected (Table 4);

(d) a herd probably treated previously with trivalent FMD vaccine (Table 5).

In 1964 and 1965, it was difficult in the Teheran area to find groups of cattle free of antibodies against Types A and O. More recently, this has been somewhat easier due to more limited prevalence of FMD in 1966 and 1967. Type SAT 1 was last detected in Iran late in 1964 (3).

Serums recorded in Table 2 were collected in 1965. In test 1, serums from 3 bulls (Nos. 4648, 4654 and 4656) were free of specific antibody as indicated by both NT and ICF. These animals were selected for an experiment with Type A virus. The remaining cattle were rejected since their serums contained A anti-

Table 2

Screening of cattle serums by NT and ICF:
(a) Majority animals previously exposed to infection

Test No.	Cattle No.	NI			Antigen titration			Serum titration		
		A	O	SAT 1	A	O	SAT 1	A	O	SAT 1
1	4648	0	0.7	.	38	80	51	100	100	100
	4654	0	-0.7	.	51	82	57	100	100	100
	4656	0	-0.2	.	39	71	50	100	100	100
	4646	3.0	0	.	4	66	67	86	100	100
	4649	3.7	0.7	.	0	72	57	72	100	100
	4650	3.7	0	.	0	57	37	80	100	100
	4652	5.5	0	.	0	60	63	39	100	100
	4653	4.0	0	.	0	49	42	70	100	100
	4655	5.5	1.0	.	0	73	68	42	100	100
	3494	4.0	1.2	.	0	42	46	49	100	100
	3496	4.2	1.2	.	0	67	63	72	100	100
	4645	3.5	0	.	0	37	29	42	100	100
	3497	2.0	-0.2	.	4	59	54	90	100	100
	3500	2.0	1.2	.	4	52	44	90	100	100
	2149	3.2	-0.2	.	0	30	27	51	100	98
	- control	-1.0	-1.2	.	54	77	66	100	100	100
2	4612	5.0	> 5.9	0.2	0	0	76	58	66	100
	4613	-0.2	3.2	2.0	0	2	10	37	84	91
	4615	4.0	2.4	3.7	0	0	4	41	62	83
	4618	4.5	5.2	1.0	0	26	82	80	97	100
	4657	2.0	2.0	1.0	12	30	48	97	100	100
	4658	4.3	2.0	> 4.5	0	0	8	71	78	80
	3419	4.0	1.0	4.2	0	21	15	79	100	100
	- control	-1.0	-1.0	0	41	50	51	100	100	100

• = not tested

body which probably accounted also for suspicious NI (Type O) in Nos. 3494, 3496 and 3500 and for relatively low mean fixation (MF) values (O and SAT 1) in Nos. 4645 and 2149 (5, 6). Neutraliaztion and ICF results are in good agreement.

Of 7 serums examined in the second test, two (Nos. 4615 and 4658) neutralized virus of all three types. Serums 4612, 4618 and 4657 reacted with Types A and O, serum 4613 with Types O and SAT 1 and serum 3419 with Types A and SAT 1. Neutralization and ICF results agreed qualitatively in serums 4612, 4615, 4618, 4557 and 4658, whereas ICF crossed over with the third (not neutralized) type in serums 4613 and 3419. Even though there was no close agreement with neutralization, ICF recognized all antibody-containing serums.

Table 3 gives results of screening tests with serums obtained in 1967 from herds not recently infected with FMD.

In test 1, neutralizing antibodies for Types A and O were not detected. Equivocal results in No. 3783 (Type A) and No. 3784 (Type O) were probably due to non-specific neutralization inhibitor. Three serums (Nos. 3782, 3783 and 3784) contained ICF inhibitor which was strongest in 3782 and reacted with all three antigens. Serum 3788 possibly contained weak inhibitor. The low MF value (SAT 1) in 3787 cannot be accounted for. It is unlikely that any of these animals

Table 3

Screening of cattle serums by NT and ICF:
(b) Serum donors not previously exposed but some carrying inhibitor

Test No.	Cattle No.	NI		Antigen titration			Serum titration		
		A	O	A	O	SAT 1	A	O	SAT 1
	3781	0.2	1.0	44	57	23	100	100	100
	3782*	0.2	1.0	0	0	0	26	36	41
	3783	1.2	1.0	7	21	4	94	98	99
	3784	-0.5	1.7	2	2	8	86	89	91
	3785	0	1.0	45	58	32	100	100	100
1	3786	-1.0	1.0	44	67	36	100	100	100
	3787*	-0.5	1.0	41	48	12	100	100	98
	3788	0.5	0.2	24	41	13	98	100	98
	3789*	0.2	1.0	44	57	21	100	100	100
	3790*	0.5	0.2	36	62	19	100	100	99
	-Co.**	-0.2	-0.2	46	63	29	100	100	99
	4755	0	0	78	96	62	100	100	100
	4756	1.0	2.0	71	96	54	100	100	100
	4757	0.7	0.7	68	98	46	100	100	100
	4758	0	0.2	71	96	48	100	100	100
	4759†	-0.2	0.7	80	98	51	100	100	100
	4791	0	-0.2	87	98	49	100	100	100
2	4792	0.5	0.7	68	87	50	100	100	100
	4793	0.5	0.7	62	98	49	100	100	100
	4794†	0	-0.2	78	98	60	100	100	100
	4795	0	0	78	96	59	100	100	100
	4796	0	0	83	98	60	100	100	100
	4797	0	0	81	100	48	100	100	100
	4798†	3.0	1.7	71	98	46	100	100	100
	4799†	1.0	1.2	17	0	0	80	63	74
	4800	-0.2	0.7	62	62	28	100	100	98
	-Co.	0	-0.2	73	98	62	100	100	100

* susceptible to Type 0 (generalized FMD)

** negative control

\+ susceptible to Type A (gen. FMD)

still carried maternal antibody for SAT 1. Sheep blood cells were agglutinated by serums 3782 and 3784 and to a lesser degree by serum 3783. The other serums failed to hemagglutinate. Cattle Nos. 3782 (also recorded in Table 1), 3787, 3789 and 3790 proved fully susceptible to Type O.

In test 2, more potent antigens were used than in test 1. Serum 4756 slightly neutralized Type O. This was no doubt due to neutralization inhibitor since the same serum did not react with O antigen in ICF. Serum 4798 neutralized Type A and had a suspicious NI for Type O. Another serum sample obtained from the same animal one week later neutralized only 1.5 log. of A virus (see Table 1).

ICF gave negative reactions in all cases except 4799 and 4800. These animals carried ICF inhibitor, strong in 4799, weak in 4800. Both serums agglutinated sheep blood cells, but this was also the case with serum 4791, which was inhibitor-negative in this and subsequent tests. Cattle 4759, 4794, 4798 and 4799 were shown to be fully susceptible to Type A.

Table 4

Screening of cattle serums by NT and ICF:
(c) Serums free of antibody and strong inhibitor for ICF

Test No.	Cattle No.	NI		Antigen titration			Serum titration		
		A	O	A	O	SAT 1	A	O	SAT 1
1	62 *	1.0	0.7	31	54	24	100	100	98
	63 *	0.2	0.7	23	39	19	100	100	98
	64 *	0	0.7	41	50	22	100	100	99
	65 *	0.5	0.7	44	53	27	100	100	100
	66 *	0	0.7	30	43	12	100	100	97
	67 *	1.2	2.0	30	49	12	100	100	97
	68 *	0.2	0.7	26	43	22	100	100	99
	69 *	0.2	2.0	35	54	26	100	100	99
	- co.	- 1.0	- 1.2	28	48	21	100	100	100
2	72	- 0.5	0.5	67	54	28	100	100	99
	73	0	0	68	59	56	100	100	100
	74	0	0.2	57	54	51	100	100	100
	75	0	- 0.5	63	66	54	100	100	100
	76 *	0.5	- 0.2	69	56	53	100	100	100
	77	1.2	0.5	61	47	54	100	100	100
	78	1.0	0.7	58	54	59	100	100	100
	79	0.5	- 0.2	62	49	56	100	100	100
	80 *	0	- 0.2	60	65	69	100	100	100
	81	- 0.5	0.2	67	62	49	100	100	100
	- co.	0.2	0.2	68	56	65	100	100	100

* susceptible to Type O

Table 5

Screening of bovine serums by NT and ICF:
(d) Serums giving equivocal results

Cattle No.	NI Type O	Antigen titration			Serum titration		
		A	O	SAT 1	A	O	SAT 1
20	2.2	2	0	1	88	62	88
23	1.7	29	24	19	98	78	99
24	> 3.5	1	28	20	88	99	
25	2.2	34	20	24	99	88	97
27	2.2	0	0	0	83	58	84
28	2.2	12	0	38	94	57	100
32	- 0.7	0	43	1	72	87	88
35	1.2	44	0	27	100	66	99
36	2.2	12	0	40	94	51	100
38	1.7	3	6	1	90	80	88
- Co.	- 1.2	50	96	42	100	100	100

Batches of serums collected in 1967 and giving essentially negative reactions in both tests are recorded in Table 4.

In test 1, serums 67 and 69 slightly neutralized Type O virus. This was no doubt due to neutralization inhibitor since both animals were fully susceptible to infection with Type O and reactions in ICF were negative. ICF gave no evidence of inhibitor in any serum. SAT 1 antigen used was relatively weak. In this test, ICF indicated better than NT that all animals examined were fully susceptible to Type O.

An example of clean reactions by nearly all serums in presence of antigens of proper strength is test 2. The only exception is No. 72, which yielded a low MF value with antigen SAT 1. It is doubtful whether this and the suspicious NI for Type A in No. 77 were due to specific antibody.

At the farm from which serums recorded in Table 5 were collected in November, 1966, information on past infection with FMD or vaccination could not be obtained. All animals, except No. 32, carried neutralizing antibodies for Type O, most of them to low titer. Due to insufficient quantity, the serums were not tested against other virus types. In ICF, several serums reacted with antigens of all three types. None of the animals were considered for purchase.

Attempts to eliminate ICF inhibitor

In preliminary experiments, inhibitor could not be removed by the following procedures without lowering the antibody titers of bovine immune serums tested in parallel:

(a) absorption with intact washed sheep blood cells;
(b) absorption with trypsinized and washed BHK cells;
(c) adsorption on kaolin or aluminium hydroxide;
(d) treatment with fluorocarbon (Arcton 63), chloroform, potassium periodate or trypsin.

Discussion

Data given in Tables 2 to 4 show that ICF can be used as a screening method in searching for susceptible cattle in the field. It requires less time and material than NT and is easier to carry out. Serums of animals to be selected should not differ appreciably from known normal controls in their reaction with antigens of various virus types. There will always be minor discrepancies between results obtained with different normal serums since neither ICF nor NT as routinely practised is strictly quantitative (6). It may be possible in the future to reduce variation of MF values obtained with different normal bovine serums by better adjustment of complement. Normal control serums should be critically tested before use and antigens checked by positive control serums (these were omitted in the tables). Cattle whose serums closely resembled well-known negative controls in ICF have always proved susceptible.

Results of ICF and NT agreed reasonably well in former tests (6) and those described above except with serums containing inhibitor, either for ICF or neutraliaztion. Present evidence indicates that ICF and neutralization inhibitors are distinct (Table 1). Neither kind of inhibitor protected serum donors from generalized FMD. In spite of this finding, cattle carrying ICF inhibitor should not be selected for experiments in which ICF will be used to study antibody response (6).

ICF inhibitor is not type-specific. This property distinguishes it from specific antibody provided serums under test do not contain antibodies for several virus types. This was one reason why SAT 1 was included as an indicator antigen in screening tests.

Serums containing ICF inhibitor in large or moderate amounts always agglutinated washed sheep erythrocytes, but hemagglutination was also recorded for several other normal serums (including No. 4791 in Table 3) which gave no evidence of ICF inhibitor. A possible correlation between hemagglutination and inhibitory effect remains to be investigated in more detail.

Little is known about the nature of ICF inhibitor. One present working hypothesis is that heterophile antibodies are involved which agglutinate sheep celss and react non-specifially with FMD antigen in such a way that it no longer fixes complement in presence of type-specific guinea pig immune serum. Forssman antibody may not be responsible since, as just mentioned, sheep erythrocytes are occasionally agglutinated by normal cattle serums that do not contain ICF inhibitor. The increase of ICF inhibitor by infection with FMDV (Table 1), which may act as a non-specific antigenic stimulus for cells producing hypothetical heterophile antibodies, would support this hypothesis.

Another possibility is that antibody against another virus which may have some antigen in common with FMD, plays a part. If so, an antigen which is not type-specific ("group antigen" [5], "VIA antigen" [1]) would be involved. In this case, infection with FMDV might also stimulate production of hypothetical antibody inhibiting ICF. Appearance of ICF inhibitor in a normal animal (No. 54, Table 1) not previously showing it may be a result of infection with this hypothetical virus. This theory would also explain differences observed in occurrence and frequency of ICF inhibitor in different herds of cattle.

Cattle immune to one virus type or subtype can be recognized by ICF (6; Test 1, Table 2 above). However, not enough information is available on the behavior of serums from animals immune to several virus types (test 2, Table 2). In some such cases, antibodies may cross over more strongly with other types and it may be difficult to distinguish in ICF between antibody and strong inhibitor by testing with antigens of a virus type against which the cattle population of the country is not immune.

The danger of spreading such a type accidentally in the country can be reduced by using antigens heated at 60° for 30 minutes. Such heating inactivates the bulk, if not all, of the virus and leaves CF antigen active.

Palacios and Rodriguez (2) reported briefly that many bovine serums obtained from the field produced partial fixation in ICF, which made reading of results extremely difficult. A similar test, not easy to interpret, is shown in Table 5. The assumption that the respective cattle were treated with trivalent A-O-SAT 1 vaccine some time previously would explain the result. Such vaccine was actually distributed by Razi Institute until June, 1966, and may have been stored for additional short periods at depots in Teheran and in the provinces before application. The animals were bled in November, 1966.

We wish to mention here briefly that ICF has also been used with encouraging results for screening sheep serums, which rarely contained non-specific ICF inhibitor. There is evidence suggesting that ICF is slightly more sensitive than NT in detecting specific antibody in ovine serums.

Summary and Conclusions

In a search for susceptible cattle in the field, bovine serums were screened by indirect complement fixation (ICF and parallel neutralization tests (NT) in tissue cultures. Results of both tests agreed reasonably well. ICF was suitable as a simple and rapid screening procedure saving time, material and labor.

Due to non-specific inhibitors, both tests sometimes gave equivocal results. ICF inhibitor, which was present in disturbing concentration in 12 (= 8%) of 148 serums subjected to both tests, appeared to be distinct from neutralization inhibitor. Neither inhibitor made serum donors resistant to infection with FMDV.

In contrast to specific antibody, the effect of ICF inhibitor was not type-specific. To recognize inhibitor, serums were tested with SAT 1 antigen besides A and O, since SAT 1 antibody was no longer to be expected in the younger cattle population in Iran.

Preliminary attempts to eliminate ICF inhibitor by simple procedures, without reducing antibody titers of bovine immune serums in parallel tests, were unsuccessful.

Two hypotheses concerning the possible nature of ICF inhibitor are discussed.

Acknowledgements

We thank Dr. M. Kaveh, Director General of Razi Institute, for placing facilities of the FMD Laboratory at our disposal, Miss F. Kesting and Mr. G. K. Kanhai for carrying out the neutralization tests, and Mr. Kanhai for managing the serum collected and excellent technical assistance in experiments with cattle.

Zusammenfassung

Indirekte Komplementbindung bei Maul- und Klauenseuche

II. Überprüfung von Rinderseren

Bei der Suche nach empfindlichen Rindern wurden die Seren mittels der indirekten Komplementbindung (IKB) und des zum Vergleich ausgeführten Neutralisationstestes in Gewebekulturen überprüft. Die Ergebnisse beider Teste stimmten einigermassen gut überein. Die IKB war als einfache und schnelle Prüfungsmethode geeignet; sie spart Zeit, Material und Arbeit.

Beide Methoden ergaben als Folge unspezifischer Inhibitoren gelegentlich zweifelhafte Resultate. Der IKB-Inhibitor war bei 12 (= 8%) von 148 Seren, die beiden Tests unterzogen worden waren, in störenden Konzentrationen vorhanden und schien von dem Neutralisations-Inhibitor verschieden zu sein. Keiner der Inhibitoren machte die Serumspedner gegenüber einer Infektion mit dem MKS-iVrus resistent.

Im Gegensatz zu spezifischen Antikörpern war die Wirkung des IKB-Inhibitors nicht typspezifisch. Zur Bestimmung des Inhibitors wurden die Seren neben den Antigenen A und O auch mit SAT 1 überprüft, da SAT 1-Antikörper bei der jüngeren Rinderpopulation im Iran nicht mehr zu erwarten sind.

Vorläufige Versuche, den IKB-Inhibitor durch einfache Massnahmen auszuschalten, ohne dass der parallel überprüfte Antikörpertiter von Rinder-Immunseren sich vermindert, hatten keinen Erfolg. Zwei Hypothesen über die mögliche Natur des IKB-Inhibitors werden diskutiert.

Résumé

Fixation indirecte du complément lors de fièvre aphteuse
II. Examens de sérums bovins

Lors d'une recherche d'animaux positifs, on examine les sérums à l'aide de la fixation indirecte du complément (FC) et, à titre comparatif, à l'aide du test de neutralisation sur cultures de tissus. Les résultats des 2 tests concordent assez bien. La FIC est indiquée pour un examen simple et rapide; elle épargne temps, matériel et traival.

Les 2 méthodes donnent à l'occasion des résultats dounteux, à cause de la présence d'inhibiteurs non spécifiques. On trouve l'inhibiteur de la FIC à des concentrations gênantes dans 12 sur 148 sérums (8 %) soumis aux 2 tests. Il semble se différencier de l'inhibiteur de neutralisation. Aucun des inhibiteurs ne parvient à rendre les donneurs de sérums résistants à une infection par le virus de la fiévre aphteuse.

A l'encontre des anticorps spécifiques, l'action de l'inhibiteur de la FIC n'est pas spécifique du type. Pour déterminer l'inhibiteur, on teste les sérums avec les antigènes A et O, puis également avec SAT 1, car on ne s'attend guère à trouver des anticorps SAT 1 dans les populations de jeunes bovins de l'Iran.

Les expériences préliminaires pour éliminer l'inhibiteur de la FIC par des méthodes simples, sans pour our autant diminuer le titre des anticorps des immunsérums bovins examinés parallèlement, demeurent jusqu'ici sans succès. On émet deux hypothèses sur la nature probable de l'inhibiteur de la FIC.

REFERENCES

1. COWAN, K. M. and J. H. GRAVES, 1966: A third antigenic component associated with foot-and-mouth disease infection. Virology, **30**, 528—540. • 2. PALACIOS, P., and R. RODRIGUEZ, 1949: L'épreuve de déviation du complément dans l'étude des virus aphtheux. H. GILBERT, 1966: Rapport sur l'évolution de la fièvre aphtheuse en Iran. Bull. Off. Intern. **65**, 333—336. • 4. REED, L. T., and H. MUENCH, 1938: A simple method of estimating 50 per cent endpoints. Am. J. Hyg. **27**, 493. • 5. RICE, C. E., and J. B. BROOKSBY, 1953: Studies of the complement-fixation reaction in virus systems. V: In foot-and-mouth disease using direct and indirect methods. J. Immunol. **71**, 300—310. • 6. TRAUB, E., M. HESSAMI and A. SHAFYI: Indirect complement fixation in foot-and-mouth disease. I. Study of antibody response in cattle and sheep. Zbl. Vet. Med. (in press).

Anschrift: Professor Dr. E. Traub, Institut für Mikrobiologie und Infektionskrankeiten der Tiere, 8 München 22, Veterinärstr. 13.