Behavior of Foot-and-Mouth Disease Virus on Serial Passage in Different Kinds of Cells. A Contribution to Experimental Epidemiology at Cell Level*)

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With 2 figures

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Attenuation of Iranian strains of foot-and-mouth disease virus (FMDV) for cattle by serial passage in different kinds of cells of hamster, cattle and sheep origin was reported in an earlier communication (15). The limiting-dilution (LD) method was used according to which transfer is made from cultures infected with the highest infective dilution of the preceding passage. At the time of transfer, such cultures often showed one small focus of infection only and the amounts of virus transferred were correspondingly small. This procedure is comparable with natural infection chains in the field, where this highly contagious virus is frequently transmitted in very small quantities.

In the course of this work with cells, observations were made which may have a bearing on certain epidemiological events in the field. The epidemiological phenomena under consideration are:

a) natural retrogression and termination of epizootics;
b) increase in potency of the causative agent;
c) virus adaptation to certain species of animals and escalation or decrease of infectivity and pathogenicity for other species;
d) different affinity of virus strains for certain cells (cardiac FMD);
e) differences in susceptibility among individual animals of the same species;
f) reduced susceptibility in undernourished individuals;
g) different behavior of different virus types;
h) persistent infection;
i) serological variation.

In this paper, an attempt will be made to correlate results obtained in cell cultures with these epidemiological features of the natural disease.

Materials and Methods

Virus

Strains A "Teheran", O "Rey" No. 4 and SAT 1 "Shiraz" (17) were used in this work. One small experiment only was carried out with Type Asia 1, which reappeared briefly at Mashad and at Razi Institute in 1964.

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Cell cultures

The following growth media were employed:

1. Hanks' medium with Difco yeast extract (YE) containing per liter: 960 ml Hanks' balanced salt solution (modified); 5 gms. lactalbumin hydrolysate; 0.5 gms. yeast extract; 40 ml. normal bovine serum, heated at 56 C. for 30 minutes; 100,000 units penicillin; 0.1 gm. streptomycin (1).

Modified Hanks' solution contains (gms./liter): NaCl — 8.0; KCl — 0.4; $MgSO_4 \cdot 7\,H_2O$ — 0.2; $CaCl_2$ — 0.28; $Na_2HPO_4 \cdot 2\,H_2O$ — 0.06; KH_2PO_4 — 0.06; glucose — 2.0; phenol red (water-soluble) — 0.02; $NaHCO_3$ — 0.35.

2. Hanks medium without YE containing per liter: 900 ml. Hanks' balanced salt solution; 5 gms. lactalbumin hydrolysate; 100 ml. normal bovine serum heated at 56 C. for 30 minutes; 100,000 units penicillin; 0,1 gm. streptomycin.

The salt solution contains (gms./liter): NaCl — 8.0; KCl — 0.4; $MgSO_4 \cdot 7\,H_2O$ — 0.1; $MgCl_2 \cdot 6\,H_2O$ — 0.1; $CaCl_2 \cdot 2\,H_2O$ — 0.191; $Na_2HPO_4 \cdot 2\,H_2O$ — 0.06; KH_2PO_4 — 0.06; glucose — 1.0; phenol red (w. s.) — 0.02; $NaHCO_3$ — 0.35.

VM_3 (11) was used as maintenance medium. It contained (gms./liter): NaCl — 8.0; KCl — 0.3; $CaCl_2 \cdot 2\,H_2O$ — 0.24; $MgCl_2 \cdot 6\,H_2O$ — 0.2; $NaHCO_3$ — 2.0; glucose — 1.8; lactalbumin hydrolysate — 5.0; phenol red (w. s.) — 0.01; 100,000 units penicillin; 0.1 gm. streptomycin.

All media were prepared with double-distilled water.

Cells and passage methods employed were described in the preceding paper (15). Abbreviations used for different kinds of cells are for convenience repeated here: HK = baby hamster kidney cells (primary cultures (PC) or subcultures (SC); BHK = baby hamster kidney cells (stable cell line BHK — 21); HL = baby hamster lung cells (PC or SC); BK = bovine (calf) kidney cells (PC or [rarely] first SC); BEL = bovine embryo lung cells (PC or SC); BELE = bovine embryo lingual epithelium cells (PC or SC); LT = lamb testis cells (PC or SC); BEK = bovine embryo kidney cells (PC or first SC).

The numbers of passages carried out in different kinds of cells are listed in Table 1 of the preceding communication.

We wish to mention here that several attempts to establish stable cell lines from primary cultures of HK, HL, BEL, BELE and LT cells failed. In some cases (HL, BEL, BELE, LT) it was possible to make from 20 to 40 serial subcultures and use them for virus passage, but all cell lines except BHK-21 (9) finally died out. Addition of extracts of various embryonic tissues to the growth media did not restore the original vitality of the cells. At higher passage levels, primary cultures were used predominantly.

Different lines of BHK-21 cells

The BHK cells used were originally obtained from the FMD vaccine production section of Razi Institute (Dr. M. AMIGHI) where they had been cultivated in Hanks' medium with YE (1) as described above (No. 1) Subsequently Hanks' medium without YE but with a greater amount of normal bovine serum (No. 2 above) was used as growth medium. Medium 2 had proved more suitable for primary cell cultures than medium 1 and was for convenience employed for BHK cells as well. BHK cells used for virus passage were grown in medium 2 for a period of 2 years during which 171 successive subcultures were made (line b).

In September, 1966, a new sample of BHK cells was obtained through the courtesy of Dr. AMIGHI for comparison with the above cells cultivated in the absence of YE. These cells (line a) had the same origin as line b and had been subcultured in Hanks' media with YE with similar frequency to line b.

Since Hanks' medium 1 prepared in our laboratory was slightly toxic for BHK cells of line a, a mixture of medium 1 (1 part) and medium 2 (4 parts) was used for line a.

Establishment of persisting infection

BK, bovine embryo kidney (BEK) or OEK cells were grown as monolayers with Hanks' medium 1 in 1 liter Blake bottles. Before infection with unmodified virus of Types O or SAT 1, the cells were washed with VM 3. The medium used in infected cultures was VM 3 with 2 % normal horse serum heated at 56° C. for 30 minutes. In cultures containing surviving cells, media changes were made every 4 to 14 days depending on the number of surviving cells, which varied greatly.

Infectivity tests were made at irregular intervals by inoculating test-tube cultures of OEK (primary) cells containing 1.8 ml. VM 3 with 0.2 ml. samples of undiluted culture fluid.

Complement-fixation (CF) tests

The method of TRAUB and MÖHLMANN (16) with minor modifications (17) was used.

BHK cultures in Blake bottles to be tested in CF (see Table 4) were alkalinized slightly (pH about 8.2) by adding NaOH, deep-frozen, thawed and centrifuged, using the supernatant fluid as antigen.

Passage strains were tested at intervals for type-specificity by CF. Sometimes it was necessary to concentrate the antigen by ultracentrifugation.

Results

Parallels in cell cultures for the retrogression and termination of epizootics

Virus types A, O and SAT 1 could be adapted to HK (BHK), BK and LT cells without difficulty and were readily maintained in serial passage using the LD method. Cytopathic effect was marked and regular, and infectivity titres of LD cultures were relatively high. At higher passage levels, infectivity titres dropped and it became increasingly difficult to continue the passage series.

Fig. 1 shows the behavior of Types A, O and SAT 1 in BK cells. In the curves, mean infectivity titres, each calculated for 10 successive passages, are recorded. Similar results were obtained with LT cells (6) in which all types gradually died out after some 40 serial passages in spite of the fact that they reached surprisingly high infectivity titres in earlier passages. In both BK and LT cells, direct passage series could not be reestablished by alternating passage in other cells.

The possibility had to be considered that the faltering and eventual loss of all three virus types in LT cells was due to variation in cell susceptibility (see below). Clear-cut CPE was not obtained when duplicate cultures were infected with low-passage material of the same strains. However, infection with Type Asia 1 (first passage in BK cells) in low and high dilutions always caused complete cell destruction. BK cells did show CPE when infected with low-passage material of A, O and SAT 1, which proves that the cells were susceptible.

Difficulties were also experienced with Type A in BHK cells. This passage chain broke repeatedly at high passage levels (217 to 248), but it was possible in every case to continue from indicator cultures in highly susceptible OEK cells included with every passage in BHK to ascertain the presence of virus (15). The LD method was abandoned at passage 265 and undiluted culture fluid was henceforth used for serial transfer.

To find the reason for the gradual decline of virus multiplication at high passage levels in BHK cells, two parallel passage series were initiated with Type A (starting from passage 313) and with Type O (starting from passage 328). In series I, cultures were infected with undiluted culture fluid of the preceding passage; in series II, the LD method was used.

In series I, the result was the same for both virus types. They could be passed without interruption 66 times and infectivity titres of passage cultures were high ($< 10^{-7}$ or 10^{-8}) whenever tested in OEK cells. CPE was always complete 15 to 18 hours after infection. It seemed that unlimited serial passage would have been possible with this method. The same was the case with Type SAT 1 in BHK cells.

In series II, Type A was passed uninterruptedly 61 times, but infectivity titres were much lower and CPE less than in series I. Further direct serial passages appeared possible when the experiment was terminated. In series II of Type O, the virus was lost after 12 passages and the OEK indicator culture was used to infect the cultures of the following passage in BHK. Subsequently, 46 more passages in BHK cells were made but, in view of very limited virus multiplication and poor CPE, it appeared unlikely that unlimited passage would have been possible using the LD method.

To determine whether viral interference was the cause of this behavior of passage strains in BHK cells, five experiments on interference were carried out

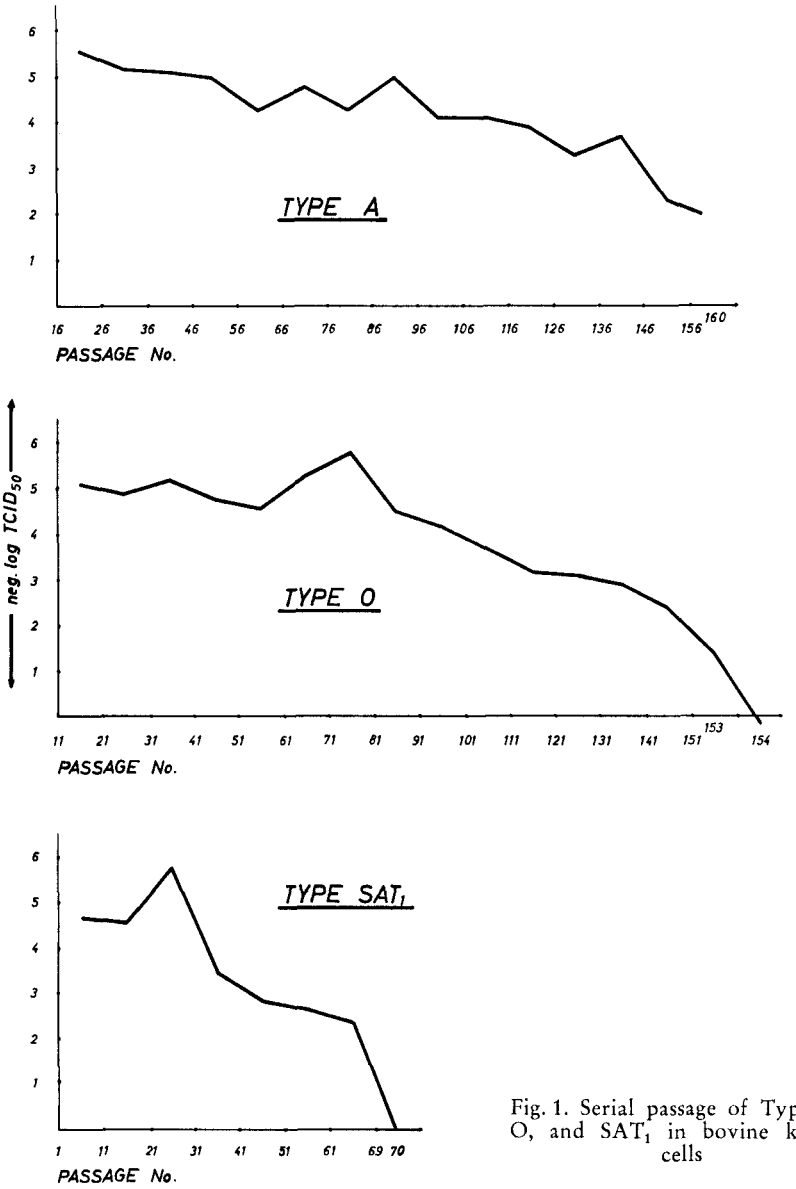

Fig. 1. Serial passage of Types A, O, and SAT_1 in bovine kidney cells

with BHK cells using virus strains of low cytopathogenicity, which did not give rise to plaques, and a highly cytopathic strain (SAT 1 — LT — BHK), which did cause plaque formation, varying dosage and time interval between infection, but we failed to get any clear evidence of interference in this system. Moreover, we have been unable to demonstrate homotypic interference between BHK passage strains of series II and I just mentioned.

Demonstration of interferon activity towards FMDV did not succeed in BHK cultures taken from passage ranges where viral multiplication and CPE were poor. Indicator viruses more sensitive to interferon than FMDV were not used because their importation into Iran appeared too risky.

More or less sudden increases in potency of the virus

This phenomenon was observed three times with different passage strains of Type SAT 1. It was missing or less marked with the two other types. The strains concerned are SAT 1 — LT — BHK, SAT 1 — BEL and SAT 1 — OEK (15).

The first strain had undergone 44 passages in LT cells and 4 in OEK cells before transfer to BHK cells. In them, virus titres were initially high but dropped to very low levels later. A sudden increase in potency occurred after passage 169. This change remained until passage 229, when the LD method was abandoned, and was still evident at terminal passage 328. The curve in Figure 2 shows passage range 151 to 198 in BHK cells.

Adaptation of Type SAT 1 to BEL cells was not easy. Between passages 1 and 50, 9 alternating passages in BK or OEK cells were required to repair breaks in the passage chain. CPE was often missing and later erratic. A permanent increase in potency took place after passage 93 (see curve in Fig. 2).

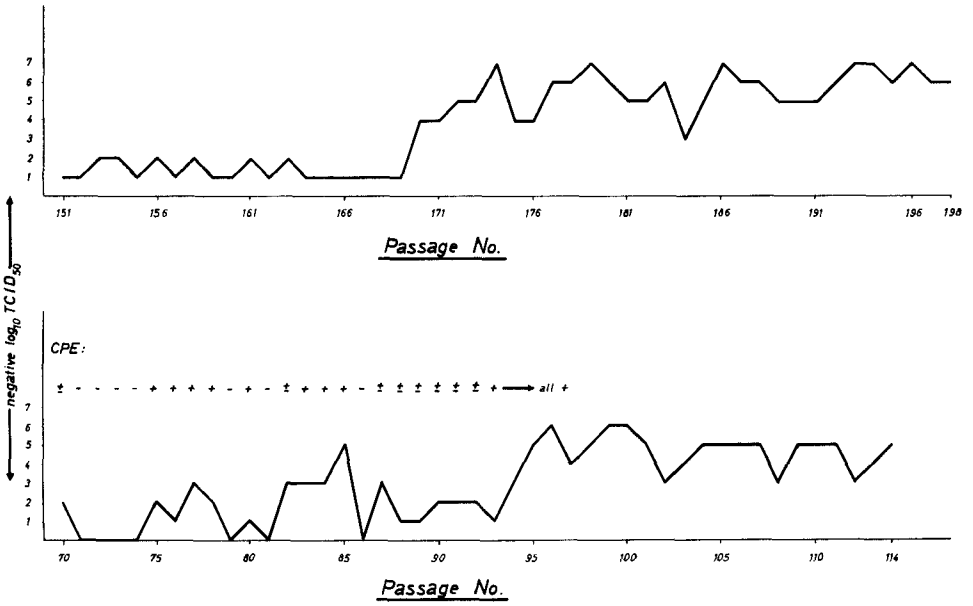

Fig. 2. Increase in virulence of SAT_1-LT-BHK (top) and SAT_1-BEL strains (bottom)

After a good start, serial passage of SAT 1 in OEK cells also caused difficulty as long as the LD method was used (up to passage 68) and virus titres became very low. Interruptions occurred after passages 42, 43 and 47. Transfer of undiluted culture fluid prevented further breaks. Cytopathogenicity increased at passage 85. CPE was complete from then on and the rate of viral multiplication was high.

In all three cases, a concurrent increase in pathogenicity for other cells, i. e., HL, BK, BELE and OEK, was noted. This change was particularly striking with strain SAT 1 — OEK, which had poor CPE in other cells at passage 32, but was very pathogenic for cells of hamster and bovine origin at higher passage levels (82 to 105).

Virus adaption to certain cells resulting in escalation or reduction of infectivity and pathogenicity for other cells

Besides the three strains just mentioned, the passage strain of Type O in BEL cells also acquired increased potency for other cells as evidenced by

higher infectivity and rate of viral multiplication and much more extensive CPE. Adaptation of Type O to BEL cells was relatively easy. CPE appeared in early passages and increased quickly. Higher virulence for other cells, first noted with BK, was already evident after 15 passages in BEL and was very marked after 30 passages. Such virus caused complete destruction of BK cells, which was rarely seen in cultures infected with unmodified strains of FMDV. However, this strain lost much of its acquired heterologous cytopathogenicity in the course of prolonged passage in BEL and OEK cells. This was most striking with virus from passage 253 when tested in HK, BHK and HL cells.

Loss of virulence for BK cells was first observed with Types A, O and SAT 1 after 30 to 40 serial transfers in HL cells. A similar change occurred later with the O — LT and O — BELE passage strains.

Rapid loss of pathogenicity of Type SAT 1 for OEK (primary) cells through passage in HL cells (made according to the LD method) is shown in Table 1. As controls in infectivity tests in OEK, primary cultures of HK cells were used, which are more susceptible to HL passage strains than BHK-21 cells. Pathogenicity for OEK cells returned briefly in passages 56 and 57 and then disappeared again.

Table 1

Changes in pathogenicity of Type SAT 1 for OEK cells through serial passage in HL cells

HL passage tested	CPE in OEK (primary) cells	HK (primary) controls
5	+	+
6	+	+
10	+	+
16 , 21 , 22 , 26 , 31 , 32 , 41 , 42 , 43 , 44 , 45 , 46 , 47 , 49 , 51 , 52	all -	all +
56	+	+
57	+	+
61 , 62 , 71 , 72 , 77 , 80 , 81 , 87 , 92	all -	all +

From passage 57 in HL, a subseries of 14 passages in OEK cells was started (15, Table 1), infecting the cultures with undiluted culture fluid of the preceding passage. Marked CPE was seen in every passage and moderate pathogenicity for BK cells returned after 5 passages in OEK.

Variation in susceptibility of cells from different animals of the same species

Such variation was frequently observed and was a handicap in attenuation work, especially as long as the LD method was used. It was perhaps most striking with BK cells, but also evident with BEL, BELE and HL cells. Differences would range from nearly complete insusceptibility to high susceptibility permitting virus multiplication to high titer and extensive or complete cell destruction.

Variation in cell susceptibility no doubt contributed to the difficulties experienced in adapting strains of FMDV to certain cells and to the failure of adaptation efforts with Type SAT 1 and BELE cells. In this case, 49 alternating passages in BELE and highly susceptible cells did not lead to permanent adaptation.

Highly potent passage strains could frequently overcome susceptibility barriers and cause extensive cell destruction in cultures of more resistant cells as well.

No marked differences in susceptibility were noted in HK and OEK cells, this being one of the reasons why highly susceptible OEK cells were used in virus neutralization tests.

As a rule, extent of CPE and rate of viral multiplication were closely correlated. Whenever CPE was missing or poor, difficulties were experienced in maintaining the respective strain in serial passage.

Variation in cell susceptibility was even more marked in subcultures than in primary cultures, especially with BK cells. BK subcultures were generally not susceptible enough to passage strains of all three types. Different lines of subcultures of HL, BEL, BELE and LT cells varied greatly in susceptibility. Some lines proved highly susceptible and gave rather regular results so that their ultimate death meant a setback. Other lines were more or less unsuitable for passage work.

Influence of cell nutrition on susceptibility and production of CF antigen in BHK cells

The influence of cell nutrition was studied using BHK cell lines a (cultivated in presence of YE) and b (cultivated without YE) as described in Materials and Methods.

Blake-bottle cultures (monolayers) of line a always showed more luxurious cell growth than cultures of line b when media were used to which the cells were accustomed. Exchange of media decreased cell growth with both lines (see Table 3).

Table 2

Parallel virus passages in two sublines of BHK-21 cells cultivated in different growth media

Passage strain	Passage No.	BHK line *	Estimated extent of CPE (%) ** Virus dilution cultures 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
A - BHK Series II	321	a	100	100	75	0	6	0		
		b	87	100	25	0	0	0		
dtto.	323	a	100	100	100	37	0	0		
		b	25	25	6	0	0	0		
dtto.	324	a	100	100	100	12	0	0		
		b	62	50	6	0	0	0		
O - BHK Series II	336	a	75	12	0	0	0	0		
		b	0	0	0	0	0	0		
dtto.	338	a	75	50	37	0	0	0		
		b	12	0	0	0	0	0		
dtto.	339	a	100	100	100	12	0	0		
		b	37	12	6	0	0	0		
SAT_1 - BHK	246	a	100	100	100	100	75	50	0	
		b	100	87	50	37	0	0	0	
dtto.	247	a	100	100	100	100	100	37	0	
		b	100	100	87	62	50	6	0	
SAT_1 LT - BHK	279	a	100	100	100	100	100	100	100	
		b	100	100	100	100	75	25	12	
dtto.	280	a	100	100	100	100	100	100	75	37
		b	100	100	100	100	100	87	62	6

*a: grown in Hank's medium with yeast extract
b: grown in Hank's medium without yeast extract
**: cultures incubated 43 to 46 hs at 37 C.

As Table 2 shows, line a was more susceptible than line b to BHK passage strains of series II of Types A and O mentioned above. In these passage series, the LD method was used and viral multiplication was relatively poor. The

difference in susceptibility was less marked towards the more virulent passage strains of Type SAT 1, especially LT — BHK, where passage was made with undiluted culture fluid. A titration experiment in which 4 tubes were used per decimal virus dilution confirmed the higher susceptibility of line a towards A and O virus of series II.

Table 3

Production of complement-fixing antigen by BHK lines a and b grown in Hanks' media with and without yeast extract

BHK line	Growth media	Culture No.	Cell growth	CPE * %	Titration of CF antigen ** Antigen						
					1 : 1	1 : 1.5	1 : 2	1 : 3	1 : 4	1 : 6	1 : 8
a	with YE †	1	very good	100	100	100	100	100	100	37	12
		2	very good	100	100	100	100	100	100	62	12
	without YE	1	moderate	100	100	87	62	12	0	0	0
		2	moderate	87	100	100	100	25	12	0	0
b	with YE	1	poor	100	12	0	0	0	0	0	0
		2	poor	87	87	50	12	0	0	0	0
	without YE	1	good	50	100	100	100	25	12	0	0
		2	good	75	100	100	100	50	12	6	0

* estimated after incubation for 21 hours at 37 C.
** figures indicate estimated fixation (%) in presence of type-specific guinea pig immune serum (1 : 10) and antigen (culture fluid, undiluted and in serial dilutions in VM_3)
† YE = yeast extract

Table 3 gives data on cell growth in Hanks' media with and without YE, extent of CPE and production of CF antigen by BHK lines a and b in 1 liter Blake bottles. These cells were infected on the fourth day of cultivation with low-passage Type A virus (second passage in BHK) and incubated for 21 hours at 37 C. Cell growth and antigen production were best in presence of the medium to which the cells were adapted, line a producing twice as much antigen as line b.

The differences between BHK lines a and b still existed after subculturing them more than 30 times in the respective media. Finally, line b was discontinued since antigen production had consistently been better in line a.

Behavior of different virus types

The three virus types under study did not behave strictly alike on serial passage in various kinds of cells. Type O could be more readily adapted than other types to BEL and BELE cells and more easily maintained in serial passage. As already mentioned, adaptation of SAT 1 to BELE cells failed. Apart from the rapidity of adaptation, there were no marked differences in behavior of the three virus types in HK, BHK, HL, BK and LT cells. Adaptation was generally slower with Type SAT 1 than with O, Type A taking an intermediate position.

Considering the behavior in all cells over the first 50 passages, Type O was, on the average, more infectious, more cytopathogenic and gave more regular results than the other types.

As already mentioned, a striking characteristic of Type SAT 1 was its tendency to escalation of potency, which was less marked with the other types.

Persistent infection

Little difficulty was experienced in establishing persistent infection with Type O in surviving BK or BEK cells. In BK cultures made in 1 liter Blake

bottles, CPE was less and the number of surviving cells greater when cultures were made from cells which had not attached to the glass in primary cultures during the first two days of incubation. In such cultures, Type O would persist with about the same frequency as in primary BK cultures made in the usual way.

Table 4
Persistent infection in cell cultures

Virus type	Cells	No. of cultures	Persistence of virus (days)
0	BK	1	125
		1	113
		2	90 - 91
		1	86
		2	35 - 41
		3	11 - 20
		5	2 - 4
	BEK	2	30
		1	6
SAT 1	BK	1	14
		2	12
		15	2 - 4

As Table 4 shows, persistent infection could be established more readily with Type O than with Type SAT 1. However, in all cultures with persisting Type O infection, the cells finally died out. The impression was gained that periods of marked viral multiplication with resulting cell destruction alternated with periods of reduced virus replication and moderate cell growth. In cultures gradually dying out, detectable quantities of virus were sometimes produced by very few surviving cells. The longest period for which virus-positive cultures could be maintained was 125 days. It is likely that maintenance for longer periods would have been possible if bovine serum had been used in the medium instead of horse serum. This was not done because Type O immunity was widespread among cattle in Iran and small amounts of antibody in the medium might have influenced the persistence of virus.

A small experiment with Type SAT 1 suggests that it may be easier to achieve persistent infection with this type in OEK cells than in BK cells, provided that there are enough surviving cells.

Serological comparison of high and low-passage virus

Five passage strains (A-BHK, O-BHK, O-BEL, SAT 1-BEL and SAT 1-LT) were selected for this test. Results of routine CF tests for type-specificity of passage strains indicated that, if serological variation through serial passage had occured at all, it would most probably be detected with these strains, which gave relatively weak reactions with potent immune serums for the original viruses in routine CF tests in spite of the fact that the cultures always showed complete CPE.

Crosswise CF tests and neutralization tests in OEK cultures (14) were carried out with serums from groups of guinea pigs immunized with the five passage strains (high passage levels) and with immune serums for the original field strains. CF tests were done in checkerboard fashion, testing undiluted antigen and serial two-fold antigen dilutions against serial serum dilutions (1 : 10, 1 : 20 etc. to 1 : 320).

Results of both CF and neutralization tests clearly showed that none of the high-passage strains had undergone a significant serological change. The results are therefore not presented in detail.

Discussion

An attempt will be made to correlate observations made in cell cultures with certain epidemiological features of the natural disease. A recent review of the epidemiology of FMD by RÖHRER (10) deals with some of the phenomena to be discussed here.

Retrogression and termination of epizootics

One of the factors responsible for the decline of epizootics no doubt is acquired specific immunity, but it is not the only cause. This can be concluded from the recent disappearance from Western Europe of Type C, which had largely adapted itself to swine. In some countries, swine were not systematically vaccinated, since use of killed-virus vaccine is of doubtful value in pigs. The disease density was not great and, in view of the rapid renewal of the swine population, susceptible pigs must have been still available to the virus when the epizootic subsided. The large panzootic in Europe caused by Type O in 1937 to 1939 is another example. At that time, vaccination was not yet practised except in a small area in Eastern Germany. It is known from our own experience that many farms escaped infection by careful application of common preventive measures. Moreover, susceptible young stock must have again been present at the end of the panzootic. Further examples are the recent disappearance of Type SAT 1 from middle-eastern countries and of Type Asia 1 from Iran.

Gradual dying out of virus on serial passage in cell cultures is certainly not due to specific immunity. There is no evidence that interference played an essential role in BHK cells. In them, breakage of passage chains was due to prolonged transfer of small amounts of virus. This procedure was successful over a long range of passages but, in the course of time, the virus lost its original potency. This was not the case when passages were made with undiluted culture fluid.

The situation is comparable to events in nature where a strongly multiplying virus, which is excreted and transferred in relatively great amounts, has a better chance to survive and cause epizootics of large proportions than a weak virus. Some investigators speak of "genius epidemicus" (10), which can be investigated at the cellular level.

Increase in viral potency

Increases in potency of strains causing epizootics do occur. An example is the causative agent of the European panzootic of 1937 to 1939, which was introduced into France with Algerian sheep. The spreading power of this virus was not unusual at first, but increased enormously later.

Increase in potency of FMD virus on serial passage in different kinds of cells is a parallel to such events in the field. It may not be a matter of chance that Type SAT 1 was involved in all three cases observed.

Increase or decrease in viral potency for cattle by passage in swine

Alternating passage in cattle and swine in the field is believed to increase the potency of FMDV for cattle (10). This reminds one of an old experiment in which an attempt was made to transmit the so-called standard strains (Riems) to cattle (16). These strains had undergone thousands of serial passages in guinea pigs. Direct infection of cattle succeeded only with the Type A

strain, whereas Types O and C required passage in swine before they could be transmitted to bovines.

Epizootics more or less confined to swine occurred in Europe from time to time. In such cases, prolonged natural passage in swine decreased infectivity for cattle.

Comparable to this is passage strain O-BEL, where passage in BEL cells first increased and later decreased virulence for BK and other cells. Another parallel at cell level is the loss of infectivity and pathogenicity of Type SAT 1 for OEK cells through passage in HL cells (Table 1).

Cardiac FMD

Myocardiotropism varies within wide limits with different strains of FMDV. It is not a stable characteristic of certain types or strains. This was shown by a minor epizootic caused by an A variant in western Germany in 1948 and 1949 where, at onset, about 80 % of the affected cattle of all ages died from cardiac FMD. There was panic among stock owners, but mortality soon returned to near normal figures. The epizootic was later effectively controlled by vaccination. A change in the opposite direction is not known from personal experience, but RÖHRER (10) mentions its occurrence.

Although we did not work with muscle cells, results obtained with other systems (SAT 1-LT-BHK; SAT 1-BEL; SAT 1-OEK; SAT 1-HL) indicate that such changes may occur randomly at cell level under constant environmental conditions.

Variation in individual susceptibility

FMDV does not always cause disease in all animals of an infected herd. Some animals contract inapparent infections followed by immunity. Others escape infection entirely and fail to develop specific antibodies. An extreme case of this sort was observed by the senior author in 1951 in Colombia, S. A., where only one ox in a herd of some 120 cattle showed severe FMD caused by Type O (O_3), which had just invaded the country from Venezuela. The team mate of this ox failed to show symptoms. Repeated visits to this farm confirmed the absence of other clinical cases of FMD. In a simultaneous outbreak on a neighboring farm caused by the same virus, morbidity was close to 100 %.

Differences in susceptibility to contact infection may be correlated with differences in cell susceptibility as described above.

Influence of nutrition on susceptibility and antigen production

It is generally agreed that cattle in a poor state of nutrition are less susceptible to FMD than well-nourished individuals (10). This is also the case in guinea pigs where formation of primary vesicles is poor and generalization often missing in undernourished individuals.

Results obtained with BHK cells originating from the same source but fed in different ways through many generations are in agreement with observations made with large animals and guinea pigs. In view of the homogeneity of these cells, it is most likely that differences in cell nutrition accounted for the different susceptibility of BHK lines a and b to certain passage strains. Cells of line b, which had a lower growth potential than those of line a, may be regarded as nutritionally deficient. Although the growth media used for these lines differed in more than one respect (see Materials and Methods), it is strongly suspected that yeast extract (offered to line a) was the decisive factor.

Chromosome studies were not made with lines a and b. If there were differences in this respect, they might have been primarily due to different cell nutrition.

The difference in production of CF antigen by BHK lines a and b can be correlated with differences in antigen content of cattle tongue epithelium in Iran and in Europe. In Iran, where many cattle are in a poor state of nutrition (we worked with such animals almost exclusively), we were often surprised by the low antigen content of bovine vesicular epithelium, which averaged only one-fourth to one-third of that generally found in well-nourished animals in Europe. This difference is certainly not due to different potency of guinea pig serums used in CF tests here and there. Decreased virus production by poorly nourished animals may in part account for the fact that the disease in Iran is generally less contagious than in Europe.

Behavior of different virus types

In the past, the most extensive panzootics in Europe (1920; 1937 to 1939) were caused by Type O. Of the 4 types encountered in recent years in Iran, Types O and A were the most tenacious in the field, Type A showing a marked tendency to serological variation (17). Types Asia 1 and SAT 1 disappeared from the country, and this was certainly not due to vaccination.

In cell cultures, adaptation and maintenance in serial passage were relatively most easy with the O strain used. Types A and SAT 1 followed in this order.

Type Asia 1 was tested in LT cells only. CPE was complete in cells resistant to Types A, O and SAT 1. From this observation one may conclude that, in Iran or Afghanistan where it came from, Type Asia 1 may have adapted itself to sheep to a greater extent than other types under study. Its contagiousness in cattle was of a low order at Mashad in spite of the presence of a cattle population fully susceptible to this type.

Persistent infection

Observations made recently indicate that FMDV may persist in convalescent animals for considerable periods of time (18, 13, 2), but there have also been negative results (19). The amounts of virus detected were very small, insufficient for spread by contact. They are presumably too small for effective heterotypic interference as well, since young animals naturally immunized to Type A were fully susceptible to contact infection with Type O (14). Affirmative reports are now on hand from Holland, Brazil and England, and there is therefore no more reason to doubt that virus carriers exist, even though they may be unimportant epidemiologically (10).

Persistent infection with Type A in surviving calf kidney cells was reported by other investigators (4, 12). We have confirmed this observation for Type O, but had little luck in establishing chronic infections with Type SAT 1. Since antibodies are not produced in cultures, conditions there are different from those in animals.

Serological variation

The way in which different virus types and variants arise in the field is still a matter for speculation. However, much progress was recently made by experiments in which new variants were produced artificially by passage of virus strains of Types SAT 1 (8) and O (5) in partly immunized cattle.

New variant strains were also recovered during passage of FMDV in monolayer cultures of pig kidney cells in the presence of strain-homologous antiserum (7).

Although a certain degree of serological variation has been reported for passage virus cultivated in the absence of immune serum (3), negative results of our tests made with five passage strains of different types using CF and virus-neutralization methods indicate that serological variants are not readily obtained by passage in the absence of immune serum.

Conclusions

In the course of serial passage of Iranian strains of foot-and-mouth disease virus (FMDV) of Types A, O and SAT 1 in monolayers of different kinds of cells according to the limiting-dilution method, observations were made which can be correlated with certain epidemiological features of the natural disease.

There were parallels for the natural retrogression and termination of epizootics. Infection of passage cultures at low multiplicity reduced viral potency and led to breaks in passage chains. Infection at higher multiplicity permitted unlimited transfer. There is no evidence that decline in viral potency in BHK cells was due to interference phenomena.

More or less sudden increases in viral potency occurred with three passage strains of Type SAT 1.

Virus adaptation to certain cells led to escalation or decrease of viral infectivity and pathogenicity for other cells, changes similar to those occurring through natural passage of FMDV in pigs.

The variable incidence of cardiac FMD in animals can also be explained by viral changes occurring at cell level.

The susceptibility of cells from different animals of the same species to certain passage strains varied within wide limits. This may reflect differences in susceptibility among individual animals as observed in the field.

Low susceptibility to FMDV in undernourished animals has a parallel in decreased growth and susceptibility of BHK cells grown in Hanks' medium without yeast extract. Reduced production of CF antigen by cells grown in deficient nutrient medium resembles the relatively low antigen content of vesicular epithelium in undernourished animals.

The capacity of Type O to cause panzootics of enormous proportions before introduction of vaccination may be correlated with the relatively higher potency of this type in cells of bovine origin compared with other virus types studied.

Persistent infection occurs both in cattle and in bovine cells.

On the other hand, serological variation, quite common in the field, could not be demonstrated in the course of serial passage of virus strains in cell cultures free of antibodies.

Summary

In the course of serial passage of Iranian strains of foot-and-mouth disease virus of Type A, O and SAT 1 in monolayers of different kinds of cells according to the limiting-dilution method, observations were made which can be correlated with certain epidemiological features of the natural disease.

There were parallels at cell level for: the natural retrogression and termination of epizootics; increases in viral potency; virus adaptation to one susceptible species leading to escalation or decrease of virulence for other species; variable incidence of cardiac foot-and-mouth disease; differences in susceptibility among individual animals of the same species; lowered susceptibility of undernourished animals; relatively higher potency of Type O compared with other virus types studied; persistent infection.

Serological variation, quite common in the field, could not be demonstrated in cell cultures free of specific antibodies.

Zusammenfassung

Verhalten des Maul- und Klauenseuche-Virus bei serienweiser Passage auf Zellen verschiedener Art. — Ein Beitrag zur experimentellen Epidemiologie auf der Zellebene

Im Verlauf serienweiser Passagen iranischer MKS-Virusstämme nach der Grenzverdünnungsmethode auf Monolayerkulturen unterschiedlicher Zellart wurden Beobachtungen gemacht, die mit bestimmten epidemiologischen Zügen der natürlichen Erkrankung in Beziehung gebracht werden können.

Auf Zellebene fanden sich Parallelen für das natürliche Zurückgehen und Erlöschen von Epizootien, das Ansteigen der Viruspotenz, die Virusanpassung an eine empfängliche Art bei gleichzeitigem Zu- oder Abnehmen der Virulenz gegenüber anderen Arten, unterschiedliches Vorkommen der kardialen MKS-Erkrankung, Unterschiede in der Empfänglichkeit zwischen verschiedenen Individuen derselben Tierart, verminderte Empfänglichkeit unterernährter Tiere, relativ höhere Potenz des Types O im Vergleich mit den anderen untersuchten Typen, persistierende Infektion.

Die in der Praxis häufige serologische Variation konnte in Zellkulturen, die von spezifischen Antikörpern frei waren, nicht nachgewiesen werden.

Résumé

Comportement du virus de la fièvre aphteuse au cours de passages sériés sur des cellules d'espèces différentes. — Contribution à l'épidémiologie expérimentale sur le plan cellulaire

Au cours de passages sériés de souches iraniennes du virus de la fièvre aphteuse, selon la méthode de dilution limite sur cultures en monolayers de différentes espèces cellulaires, on peut faire quelques constatations, qui peuvent être comparées à certains aspects épidémiologiques de la maladie naturelle.

Sur le plan cellulaire, on peut établir des parallèles avec la régression et l'extinction des épizooties, l'augmentation de la puissance du virus, l'adaptation du virus à une espèce réceptive, avec augmentation et diminution simultanée de la virulence envers d'autres espèces, l'apparition variable de la fièvre aphteuse à forme cardiaque, les différences de réceptivité entre individus de même espèce animale, une prédisposition moindre des animaux sous-alimentés, une puissance relativement plus élevée du type O, en comparaison avec les autres types examinés, une infection persistante.

La variation sérologique, souvent observée en pratique, ne peut être mise en évidence dans les cultures de tissus, qui sont dépourvues d'anticorps.

Resumen

Comportamiento del virus aftoso en pasajes seriados por células de diversos tipos. — Una contribución a la epidemiología experimental a nivel celular

En el curso de pasajes proseguidos de cepas virósicas aftosas inraníes con arreglo a la técnica de las diluciones límites en cultivos monoestratificados de tipos celulares más diversos, se realizaron observaciones que se pueden relacionar con ciertos rasgos epidemiológicos de la enfermedad natural.

A nivel celular, se hallaron paralelas para el retroceso natural y extinción de epizootías, el aumento de la potencia virósica, la adaptación vírica a una especie receptible con aumento o disminución simultánio de la virulencia frente a otras especies, presencia discrepante de la enfermedad aftosa

cardíaca, diferencias en la receptividad entre individuos diversos de la misma especie animal, receptividad disminuida de los animales desnutridos, potencia relativamente superior del tipo O en comparación con los otros tipos examinados, infección persistente.

La variación serológica, frecuente en la clínica rural, no se pudo apreciar en los cultivos celulares que estaban libres de anticuerpos específicos.

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