"HAND-FOOT-AND-MOUTH DISEASE" IN BIRMINGHAM IN 1959

BY

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In June, 1959, a child aged 3 had a painful stomatitis with fever, cervical adenitis, and a vesicular rash on hands and feet. He was referred to a consultant paediatrician (Dr. H. McC. Giles), who, recognizing that the disease was outside the usual run of children's infections, referred him to the virus laboratory. When three children similarly affected were found in the practice, the College of General Practitioners was notified and asked to help, through its Epidemic Observation Unit, to obtain reports of similar cases from other practices. In response to the "red warning" issued by the College, a total of 83 cases were notified in June, July, and early August. The "red warning" is a notification to members of the research register of

the College that a condition fulfilling certain criteria has been identified as being epidemic in a given place. All those receiving the "red warning" are invited to return information of any such cases they may see to the Epidemic Observation Unit of the College. Of these 83 cases, 24 occurred in Birmingham, most of them being seen by J. A. and/or T. H. F. We were able in the other cases, by discussing them with the practitioners reporting them, to be reasonably sure that the patients described had a disease similar to that which we had ourselves seen. A few other cases were reported to us by lay witnesses; their children had had such a mild illness that the parents had not thought it worth while to call their doctor. The description below is based upon the cases seen by us.

Clinical Features

The disease was mild and in most cases lasted less than a week. There were no prodromata, and the presenting feature in all but two cases was a painful stomatitis. One patient (the oldest, aged 25) had lesions upon her hands only. Skin lesions were present in 19 cases, and two children had cervical adenitis and fever up to 100 and 101° F. (37.8 and 38.3° C.) respectively for one or two days.

The lesions in the mouth were irregularly shaped shallow ulcers varying from 1 to 10 mm. across, with a yellowish-grey base and hyperaemic margin. They occurred on the tongue, usually on its sides, on the inside of the cheeks, on the anterior fauces, on the mandible above the posterior molar tooth, and on the soft palate. They were not usually very numerous, but were painful at first and in some children caused difficulty in eating. Lesions were not seen on the outside of the lips or on the pharynx or tonsils. Although by the time we saw them the mouth lesions had always ulcerated, we were told by several independent lay witnesses that the mouth lesions had begun as vesicles.

Eighteen patients had vesicular skin lesions. The blisters were thin-walled and superficial, containing a clear fluid. They varied from 1 to 5 mm. across, and were irregular in shape, usually being oval rather than round. The blisters were usually pearly grey, thin-walled, and superficial, containing a clear serous fluid, and were surrounded by a zone of erythema about 1 mm. wide. The eruption was not profuse, and only three or four vesicles might be present in any one patient. They were not painful or itchy, and the contents were absorbed in two to four days; the lesions appeared in the later stages of absorption as cherry-red

FIG. 1.—Vesicular lesion on heel.

FIG. 2.—Vesicular lesions on palm of an adult.

FIG. 3.—Maculo-papular rash on buttocks.

spots. The vesicular rash was confined to the hands and feet, occurring mainly on the dorsum of the fingers and toes near the nail margins, and also on the heels (Fig. 1). They were not often seen on the palms and soles, but when they did occur they were more profuse, smaller, and more uniform in size, resembling small grains of rice. The one adult, who had superficial vesicles on her palms only, complained that the lesions were painful (Fig. 2). One child had a vesicle on the lobe of one ear in addition to those in the mouth and on the hands and feet. Four children had a maculo-papular rash on the buttocks (Fig. 3). Rashes are common on the buttocks in young children, but we think that this rash may have been part of the disease, because in its appearance and disappearance it coincided with the other lesions. Some parents reported a fleeting faint pink generalized rash, but this had gone by the time we saw the patients.

Epidemiology

The age distribution is shown in Table I.

The illness seemed to be highly infectious in the age-group principally affected, with a fairly constant incubation period of five to six days. Although on

TABLE I.—Age Distribution and Salient Features of Cases Seen in the Birmingham Area in 1959

Age	No. of Cases	Stomatitis	Exanthem	Other Features
0-4 years ..	14	14	10	Two children had cervical adenitis and fever
5-9 " ..	7	7	6	
Over 9 " ..	3	2	3	
Total ..	24	23	19	

several occasions the illness could be traced from brother to brother, or from neighbour to neighbour, it is interesting to note that an isolated case occurred in a residential nursery where there were 20 children under the age of 5 years, and where the matron—a trained nurse—had been asked to keep a look-out for the disease. The occurrence and transmission of the disease are shown in Fig. 4. We saw patients in houses of every social class and in every grade of residential area, from the best of Solihull to the worst of Smethwick.

FIG. 4.—A diagrammatic illustration of the occurrence and transmission of the infection. The arrows indicate apparent transmission from case to case by contact; dotted lines represent transmission reported to us; continuous lines refer to cases we ourselves saw.

Virological Investigations

Culture Methods.—Faeces, swabs from the lesions in the mouth, fluid from vesicles, and acute phase and convalescent sera were taken for examination from five patients and single sera from two others, and also from as many of their mothers, fathers, and siblings as were willing to co-operate or who were of an age to yield blood without excessive difficulty. The supernatant fluids from about 20% w/v suspensions of faeces which had been centrifuged at 10,000 g for 20 minutes were inoculated into suckling mice by both intracerebral and subcutaneous routes, and were also inoculated into tissue cultures of human embryo-liver cells, embryo-rabbit-kidney cells (the HuLi and ERK1 lines of Westwood, Macpherson, and Titmuss (1957) and into human amnion and monkey-kidney-tissue cultures (the "untransformed" MK1 line of Westwood *et al.*). Some conventional baboon-kidney cultures of second and third passage was also inoculated. The cultures were not rolled. Subsequently attempts were made to infect roller tube cultures of human neonatal kidney incubated at both 33 and 35° C. using both Hanks solution and "199" solution as media.

Results.—No evidence of virus activity was found in any tissue culture, even after one or two subcultures. The faecal extracts from each of the children investigated who had the lesions and vesicle fluid aspirated from lesions on the hands of the adult caused flaccid paralysis in at least one suckling mouse, and in most cases all of the suckling mice, between the fifth and the eighth day after inoculation. This appeared in the first instance in one or two limbs only, though histological examination of some of these mice showed a generalized myositis, involving especially the intercostal muscles. All but two mice, which were allowed to recover, were killed at this stage. A clarified suspension of the ground-up carcasses was inoculated subcutaneously into further litters of suckling mice; by the fifth passage all mice were paralysed within three days. All attempts to isolate virus from faeces or to cultivate the prototype A 16 strain in tissue culture were unsuccessful.

Serological Investigations

Well-grown adult mice were bled out after being immunized by the method of Dalldorf and Sickles (1956).

To prepare an antigen for complement-fixation tests, 20% suspensions in normal saline of carcasses of suckling mice which had been paralysed at the fifth passage of an isolation from one of our patients were mixed with an equal quantity of the fluorocarbon "arcton 63" (I.C.I. Ltd.) and homogenized in aliquots of about 7 ml. in an M.S.E. masticator at maximum speed for five minutes; during this time the glass containers were cooled with chopped ice. A second treatment of the suspension produced a less hazy antigen, though its antigenic activity was neither enhanced nor diminished. However, a cross reaction to low titre (1:16) given by Cocksackie A 7 antigen with a hyperimmune serum prepared against one of our isolations, which was present after a single arcton extraction, was removed by the second treatment. The supernatants, after 10 minutes' centrifugation at 1,500 g, were in most cases further centrifuged in the Spinco Model L "40" rotor at 40,000 r.p.m. for three hours. The deposits were resuspended

in their own supernatants to one-quarter of the original volume. Some carcasses of mice partly paralysed by the inoculation of faecal suspensions were similarly treated to see whether there was at this stage enough virus in the carcass to provide a satisfactory complement-fixing antigen.

Neutralization tests were performed in suckling mice according to the method of Dalldorf and Sickles (1956).

Results

As Robinson, Doane, and Rhodes (1958) had described a similar outbreak in Toronto from which they isolated a virus serologically similar to Cocksackie A type 16, we put up a neutralization test against a virus (strain P) isolated from one of our patients and also against the prototype Cocksackie A type 16 virus, using the hyperimmune serum we had prepared. No significant difference was found between the two viruses; each was neutralized so that half the mice were paralysed with a dilution of 1:50 of serum. Both the first and second sera of this patient neutralized at a dilution of 1:5 the virus isolated from him (strain P) and also the prototype A 16 virus. A difference of at least fourfold in titre of neutralizing antibodies between the acute and convalescent phase sera of another child was demonstrated against both strain P and the Cocksackie A 16 prototype viruses; in each case the acute phase serum failed to protect completely when diluted 1:5; the convalescent serum gave complete protection at 1:20. Complement-fixation tests gave the results shown in Table II.

Some of the sera show a clear rise of antibody titre; others show a surprisingly high titre early in the course of the disease. This suggests either that the children with a high titre early in the course of the disease had had a previous infection by a serologically related virus, or that the incubation period was in fact much longer than it appeared to be.

TABLE II.—Complement-fixation Titres of Sera from Patients and Contacts Against an Antigen Prepared from Prototype Cocksackie A 16 Virus. The Figures in Parentheses refer to the Interval in Days from the Onset of Disease at Which Sera were Taken

Serum	Antigen			
	Prototype A16 1:64		Strain P >1:128<1:256	
Hyperimmune Mouse Serum against Strain P	Acute	Convalescent	Acute	Convalescent
Patient				
1 (from whom strain P was isolated)	>1:16<1:32 (2)	>1:8<1:16 (16)	1:64	>1:64<1:128
2	Negative	1:64 (15)	1:16	1:256
3	1:16 (3)	1:32 (17)	1:32	1:128
4	1:32 (10)	1:16 (21)	1:64	>1:32<1:64
5	1:32 (18)	1:32 (30)	1:64	>1:64<1:128
6	>1:16<1:32 (4)	Single serum	1:16	—
7*	Single serum	Negative		Negative
8*	" "	" "		" "
9*	" "	" "		" "

* These sera were collected from contacts.

TABLE III.—Complement-fixation Titres Given Against a Hyperimmune Serum, Prepared Against Serum P, by Antigens Prepared from Suckling Mice Inoculated with Material from Patients

Case No.:	Antigen from Mice Infected by Material from Patients									A16 Prototype
	1	2	3	4	5	6	7	8	9	
Hyperimmune serum against strain P	1:64	1:256	1:64	1:128	>1:32<1:64	1:64	1:128	>1:32<1:64	>1:128<1:256	>1:32<1:64

In order to show that all the viruses isolated were serologically similar, antigens prepared from carcasses of mice paralysed by inoculation of the various faecal supernatants were put up in a complement-fixation test against the hyperimmune serum. The results (Table III) show that the viruses are all serologically similar, and that the first-passage carcasses contain as much antigen as those from later passages.

Discussion

It is clear that the disease is essentially the same as that described by Robinson *et al.* (1958), though in Birmingham it seemed to be somewhat milder than in Toronto. The clinical features are compared and contrasted in Table IV. They found that their virus was

TABLE IV.—Comparison of the Clinical Features Shown by Patients in the Birmingham and the Toronto Epidemic

	Birmingham, 1959	Toronto, 1957
Total No.	24	60
No. of patients over 9 years old ..	3 (12%)	13 (22%)
Fever	2 (8%)	34 (56%)
Oropharyngeal lesions	23 (96%)	46 (76%)
Vesicular rash	18 (75%)	15 (25%)
Maculo-papular rash	4 (17%)	24 (40%)
Gastro-intestinal symptoms	0	20 (33%)
Respiratory symptoms	0	20 (33%)
Conjunctivitis	0	11 (18%)
Cervical adenitis	2 (8%)	0

less easily neutralized by its own immune serum than was the prototype Cocksackie A 16 virus. This is not extraordinary; other viruses, notably influenza, sometimes behave in the same way. Our virus did not show this difference. Furthermore, their viruses were isolated in tissue culture; we were unable to do this. This difference is probably explicable on technical grounds; the recent paper on the isolation of the common-cold virus by Tyrrell and Parsons (1960) illustrates the effect that a small difference in medium may make in producing a cytopathic effect.

Clinically, the lesions differ from those of herpes simplex and herpangina in occurring neither on the outside surface of the lips nor on the posterior pharyngeal wall. All attempts to isolate herpes simplex virus from these patients were unsuccessful.

It is always difficult to be sure that a particular disease is due to an associated enterovirus; these viruses are always about in the community and an occasional isolation may be made, even if only by chance. However, the similarity in clinical features to the Toronto outbreak, the regularity with which the virus could be isolated, and the isolation from a vesicle on a hand lead us to believe that this disease is indeed due to Cocksackie A 16.

The biological resemblance between the Cocksackie and foot-and-mouth-disease viruses has often been remarked upon (Burnet, 1959). It is not altogether surprising to find at least one Cocksackie virus producing lesions of a "foot-and-mouth" type distribution.

Before we had identified the viruses we isolated, Dr. I. A. Galloway, of the Foot and Mouth Research Centre at Pirbright, kindly examined sera from two patients for us, but found no evidence of any antibodies against any of the standard types of foot-and-mouth disease virus.

Summary

An outbreak of mild infection characterized by vesicular lesions in the mouth and on the hands and feet occurred in Birmingham in the summer of 1959. A few patients also had a maculo-papular rash on the buttocks. Coxsackie group A type 16 virus was isolated from vesicle fluid from the hands of one. The disease was similar to that described by Robinson *et al.* (1958) as occurring in Toronto in 1957.

Our thanks are due to Dr. D. Hobson for the cultures of baboon-kidney cells, and to Dr. J. Hotchin for the prototype coxsackie A 16 virus. The technical work described will form part of a thesis for the F.I.M.L.T. by one of us (J. R. F.). Our thanks are also due to the College of General Practitioners, whose Epidemic Observation Unit enabled us to collect as many cases as we did.

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CANICOLA FEVER IN MAN FROM CONTACT WITH INFECTED PIGS

FURTHER OBSERVATIONS

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Outbreaks of human leptospirosis due to *Leptospira canicola* affecting workers on pig farms have been reported in Israel (van der Hoeden, 1956) and in Edinburgh (Seiler, Norval, and Coghlan, 1956). That apparently normal pigs were harbouring the organisms and were probably responsible for the outbreaks in the Edinburgh farms was finally established by Coghlan, Norval, and Seiler (1957) when they reported the isolation of *L. canicola* from specimens of urine collected from pigs at one of the farms where human cases had occurred. Surveys detailed in the same report revealed from the results of serological tests that 19 out of 47 piggery workers had been infected during the years 1950-5 and that over 40% of the pigs at three farms where 12 of the cases had occurred were or had been infected. In Glasgow, Michna (1959) found serological evidence of infection by *L. canicola* in the pigs at seven different farms and succeeded in isolating the leptospires from kidneys of naturally infected pigs.

Since the 1950-5 survey further sporadic cases of canicola fever have occurred among pig contacts in

Edinburgh, and the present communication serves both to summarize the salient features of these cases and to show how a leptospiral infection in a herd of pigs may be maintained for many years, thereby constituting a hazard to any person coming in contact with the animals.

Laboratory Methods

The methods employed were essentially the same as those described previously (Coghlan *et al.*, 1957); live cultures of leptospires were used for the agglutination-lysis test.

Significant Agglutination Titres.—Titres of 1 in 100 may be indicative of past or newly acquired leptospiral infection of some kind, but in this investigation only titres of 1 in 1,000 and over are considered to be significant of present active infection in a herd of pigs. To confirm the diagnosis of human cases it was essential to show, during the course of the disease, a rise in the leptospire-agglutinating titre of the patient's serum.

Observations

With one exception all the cases dealt with here were ill enough to require hospital treatment. They were all of the meningeal type of leptospirosis; none was icteric. Since 1956 there have been 16 cases of canicola fever in the Edinburgh area, and 11 of these persons had been in contact with pigs. One was an abattoir worker engaged in slaughtering pigs, one was a child who played around a piggery, and the other nine were employed in tending pigs.

Five pig farms were involved—namely, C, D, E, F, and G, two of which (F and G) had been previously incriminated in human infections of *L. canicola*. Specimens of pig blood taken from a number of animals at each of the five piggeries at the time of the human outbreaks were found to contain antibodies to *L. canicola* in titres high enough to signify recent infection. The details of the 11 human cases are given in Table I and the results of the serological examination of the pig sera in Table II (these include some tests carried out more recently as well as at the time of the human infections).

Farm C.—In addition to the two cases of canicola fever at farm C, there was one apparently subclinical infection—Mr. U, who remained symptomless, although his serum agglutinated *L. canicola* to a titre of at least 1 in 30,000 at the time when his fellow worker, Mr. S, was in hospital suffering from a severe attack of canicola fever with classical symptoms. Serological tests revealed a 36% infection rate among the pigs.

Farm D.—The piggery owner became ill with pyrexia, polyarthritides, frontal headache, and vomiting. His serum at the time of admission into hospital failed to agglutinate *L. icterohaemorrhagiae*, and *L. canicola*. Nine days later the serum had a titre of 1 in 1,000 for *L. canicola* and 1 in 30 for *L. icterohaemorrhagiae*, and by the end of the third week the titres had reached their maximum of at least 1 in 30,000 and 1 in 300 for *L. canicola* and *L. icterohaemorrhagiae* respectively. At the same time the pigs were examined for serological evidence of infection, and it was found that 9 of the 11 sera examined had titres of 1 in 1,000 and over.

Farm E.—There were four cases of canicola fever—three in July-August, 1958, and one a year later. It should be noted that three of the patients were youths under 20 years of age who had only recently taken up