J. Hyg., Camb. (1965), **63**, 441
Printed in Great Britain

A survey of enteroviruses and adenoviruses in the faeces of normal children aged 0–4 years

A report of the Public Health Laboratory Service and the Society of Medical Officers of Health*

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(*Received* 29 *March* 1965)

INTRODUCTION

In 1957 and 1958 a survey of enteroviruses in the faeces of normal children aged under 5 years in England and Wales was made to determine the prevalence of polioviruses in the community before the full introduction of inactivated poliomyelitis (Salk) vaccine (Report, 1958; Spicer, 1961). The present survey, which was similarly designed and covered 110 local authority areas (Appendix B) was made to find out the prevalence of polioviruses after Salk vaccine had been in use for over 4 years. The survey lasted 13 months; it began on 1 June 1961, and ended on 30 June 1962, although a few specimens collected at the end of May 1961 and the beginning of July 1962 were included in the analysis.

Live attenuated poliomyelitis (Sabin) vaccine was introduced for routine immunization in place of Salk vaccine at the end of February 1962; it was therefore possible to study the distribution of enteroviruses in the community before and after Sabin vaccination began. The vaccine used was trivalent Sabin vaccine and it should be emphasized that it was given for routine immunization and not in a mass vaccination campaign. A preliminary account of the poliovirus findings was given by Galbraith (1964).

METHODS AND MATERIALS

The children

A weekly sample of the population under 5 years of age in the areas administered by the local authorities participating in the survey was obtained, and each child in the sample who co-operated in the investigation was examined once. The method of sampling was similar to that described in the report of the previous investigation (Report, 1958) the children being chosen at random from the birth register by medical officers of health or members of their health departments.

In each local authority area the weekly sample contained at least one child in each year of age, that is, a total of at least five children. After the sample was chosen,

* A full list of those taking part in the survey is given in Appendix A.

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the names of some additional children were selected from the register to replace any in the original sample who could not be contacted or for any other reason did not supply a specimen.

The specimens

Medical officers of health made arrangements for the collection of specimens. In most areas, a letter describing the objects and methods of the survey was sent to the parents of the children and their co-operation was invited. A visit to the home was then made by a health visitor or health inspector who discussed the investigation with the parents and instructed them in the collection of a sample of faeces. A specimen container was left in the household and when the specimen had been obtained it was either posted to the laboratory or taken there by one of the health department staff; in either case the specimen usually reached the laboratory within 24 hr. of collection.

Laboratory methods

It was not possible to standardize laboratory methods in the survey because of wide variation among the thirty-five participating laboratories in the facilities and staff available for the investigation.

The technique for the examination of specimens in cell cultures described in the report of the previous survey (Report, 1958) was used in the present investigation but laboratories differed in certain details. In about two-thirds of laboratories two tubes of each type of cell culture were used and in the other third only one tube was used; the volume of faecal extract inoculated into the tubes in different laboratories ranged between 0·1 and 1·0 ml. In all laboratories the tubes were observed daily for cytopathic effect for at least 7 days, and the viruses isolated were identified by the use of specific antisera prepared by the Standards Laboratory for Serological Reagents, Colindale. Unidentified viruses were investigated further in the Virus Reference Laboratory, Colindale. The strains of poliovirus isolated were not classified into wild and vaccine strains, but it is probable that all those isolated before the introduction of Sabin vaccine were wild strains and those isolated after this were nearly all vaccine strains.

Most specimens were examined in newborn mice. Litters less than 48 hr. old were inoculated with between 0·01 and 0·03 ml. of faecal extract and examined daily for 12–14 days: in most laboratories the litters were inoculated subcutaneously but in some the intracerebral route was also used. Material from mice showing evidence suggestive of Coxsackie A virus infection was sent to the Public Health Laboratory, Epsom, for identification of the virus.

The records and analysis

A record card was completed for each child who submitted a specimen. Completion of the cards was begun in public health departments where the following information was provided: (1) name of local authority; (2) name, sex and date of birth of the child; (3) date of collection of specimen; (4) whether the child was one of the original sample chosen from the birth register or was one of the additional

children selected as replacements; (5) whether or not the child had at any time received poliomyelitis vaccine, Salk and Sabin vaccines being recorded separately. The cards were sent to the laboratory with the specimens and when the laboratory results were available these were entered on the cards which were then forwarded to the Epidemiological Research Laboratory. Here they were checked to ensure that the required information had been given and the data were then summarized in a simple code on the right-hand edge of each card so that in the analysis the cards could be easily read and sorted.

RESULTS

In all, 25,600 children were examined, 13,194 of whom were males, 12,403 females and in three the sex was not recorded. This was a 3·9 % sample of the population less than 5 years of age in the survey areas recorded in the census of April 1961. The size of the sample was greater in children aged less than 3 years, 4·0 %, than in children aged 3 and 4 years, 3·7 %. This was probably because older children were more difficult to contact than younger children as was found in the previous survey (Spicer, 1961).

Table 1. *Number of specimens examined and types of laboratory examination*

Type of laboratory examination	No. of specimens examined
Total all types of examination	25,600
Tissue culture	
Total	25,589
HeLa cells	17,926
Monkey kidney cells	25,112
Human amnion	4,672
M.K.2	917
Human thyroid	707
H.Ep.2	438
Human embryonic kidney	401
Human liver	23
Human lung	4
Newborn mice	17,596

Of the 25,600 specimens, 25,589 were examined in tissue culture and 17,596 in newborn mice (Table 1). One or more different types of viruses and pathogenic bacteria were isolated from 2747 (10·7 %) of the specimens; 2035 (7·9 %) specimens were positive for viruses alone and 26 of them contained two different viruses; 651 (2·5 %) were positive for pathogenic bacteria alone and 10 of them contained two different bacteria; 61 (0·2 %) were positive for both viruses and pathogenic bacteria.

The total isolation rates per cent of each virus are given in Table 2. The unidentified agents recorded in the table are not included in the remainder of the analysis. There was no significant difference between the proportion of positive specimens from children in the original random sample and the proportion from

children who were selected as replacements. The two groups of children, the random sample and the replacements, are therefore considered together in the results.

The isolation rate of polioviruses before Sabin vaccination began was 0·86 % but for comparison with the results of the previous survey, in which examinations were made in HeLa cells only, it is necessary to express the number of poliovirus isolations in HeLa cells—117—as a proportion of all HeLa cell examinations—13,159, that is 0·89 %. The isolation rate for the corresponding time of year in the previous survey was also 0·89 %.

Table 2. *Viruses isolated and proportion of specimens positive*

Virus	Specimens: Examinations in	Specimens: No. examined	Specimens: No. and percentage positive	
Poliovirus (June 1961–Feb. 1962 before Sabin vaccination)	Cell culture	18,209	156	0·86
Poliovirus (March 1962–July 1962 after Sabin vaccination)	Cell culture	7,380	389	5·27
Adenovirus	Cell culture	25,589	128	0·50
Echovirus	Cell culture	25,589	278	1·09
Coxsackie B virus	Cell culture	25,589	196	0·77
Unidentified cytopathogenic agents	Cell culture	25,589	60	0·23
Coxsackie A virus	Newborn mice	17,596	894	5·08
Unidentified agents pathogenic to newborn mice	Newborn mice	17,596	21	0·12

Polioviruses, both before and after Sabin vaccination began, Coxsackie B viruses and echoviruses were isolated proportionately more often in monkey kidney cells than in HeLa cells; the reverse was true of adenoviruses. The highest isolation rates were obtained when both types of cells were used. Coxsackie B viruses were isolated infrequently in newborn mice and only three specimens were positive in newborn mice when cell cultures were negative. There was a higher isolation rate of echoviruses in human amnion than in monkey kidney cells, but most of the isolations were made in monkey kidney cells because these cells were used most often (Table 1). The isolation rates of these viruses isolated in cell culture were calculated, the number of specimens examined in all types of cell being taken as the denominator, so that all positive specimens could be included irrespective of the type of cells in which the isolations were made. This simplified the presentation of the results and gave isolation rates which differed little from rates calculated separately for each type of cell. The isolation rates of Coxsackie A viruses were based upon the number of specimens examined in newborn mice, but in the analysis by virus type the isolation rates of virus A 9 were calculated, using the number of specimens examined in monkey kidney cells as the denominator, because this virus type was isolated most frequently in this type of cell.

Double infections

The 26 specimens from which two viruses were isolated included 10 from which poliovirus and Coxsackie A virus were isolated. Seven of these double infections occurred before and three after the introduction of Sabin vaccination. It was not possible to assess the statistical significance of the association in the same specimen of different viruses because the number of double infections was too few.

There did not appear to be any relationship between the isolation of viruses and bacteria. There were too few double infections with viruses and salmonellae or shigellae to enable any conclusion to be drawn on the significance of their association. There were 42 specimens positive for viruses and enteropathogenic *Escherichia coli*, but none of the viruses occurred together with this bacterium more often than would be expected by chance.

Table 3. *Isolation of viruses in age groups*

	Percentage of specimens positive						
	Poliovirus before Sabin vaccination began*						
Age (years)	All children	Salk vaccinated	Not vaccinated	Coxsackie A virus	Coxsackie B virus	Echovirus	Adenovirus
Under 1	0·77	0·42	0·82	4·15	0·62	0·96	0·89
1	0·93	0·53	2·83	6·45	0·85	1·32	0·48
2	1·00	0·80	2·49	5·73	0·75	1·06	0·46
3	0·86	0·82	1·31	4·38	0·92	1·05	0·38
4	0·72	0·75	0·37	4·56	0·71	1·06	0·22
Total all ages	0·86	0·71	1·22	5·08	0·77	1·09	0·50

* June 1961 to February 1962.

Sex and age

There was no significant sex difference in the isolation rates of any of the viruses. There was no significant age variation in the isolation rate of polioviruses before Sabin vaccination began, but if the children who had not received Salk vaccine are considered alone there were striking differences among age groups (Table 3). The isolation rate was low in children less than 1 year of age, rose in the 1- to 2-year age group and then fell progressively with increase in age. The other enteroviruses showed a similar pattern, which was most noticeable with Coxsackie A viruses. The age distribution of adenoviruses was different; the isolation rate was highest in children less than 1 year of age and then declined steeply with increase in age.

Area and season

The laboratories and local authorities were grouped together in seven survey areas (Appendix B and Fig. 1). There were statistically significant variations in the isolation rates of each virus among these areas (Table 4). The variations between areas may have been due partly to differences in laboratory methods, but they

Fig. 1. Participating laboratories and survey areas.

Table 4. *Isolations of viruses in survey areas*

Survey area	Percentage of specimens positive: Poliovirus before Sabin vaccination began* Type 1	Type 2	Type 3	Coxsackie A virus	Coxsackie B virus	Echovirus	Adenovirus
North	0·88	—	0·42	3·77	0·76	1·87	0·50
East Midland	0·12	—	0·52	5·10	0·12	2·17	0·06
West Midland	0·44	0·12	0·40	5·31	0·60	0·92	1·06
East Anglia	0·16	—	0·08	3·63	0·54	0·54	0·19
London	0·17	—	0·09	6·19	1·01	0·39	0·26
South-east	0·10	0·10	0·19	5·29	1·07	0·67	0·57
South-west	2·17	0·42	0·16	6·03	1·41	1·45	0·97
Total all areas	0·51	0·08	0·27	5·08	0·77	1·09	0·50

* June 1961 to February 1962.

were probably due also to true regional variations in prevalence, because different types of the same virus showed different distributions by area.

The isolation rates of all viruses were higher in urban areas than in rural areas, and most of them fell progressively from county boroughs to municipal boroughs and urban districts to rural districts. The differences for polioviruses before the introduction of Sabin vaccine, as well as adenoviruses and echoviruses were statistically significant but for Coxsackie A and B viruses they were not. Nevertheless, the similar urban to rural gradient seen with all viruses suggests that they were all more prevalent in towns than in the country. The difference in the isolation rate of poliovirus between county boroughs and other types of local authority was greater in children under 2 years of age than in older children, suggesting infection at an earlier age in the densely populated areas.

Table 5. *Isolations of viruses in 4-weekly periods*

Four-weekly period and calendar month	Percentage of specimens positive				
	Poliovirus	Coxsackie A virus	Coxsackie B virus	Echovirus	Adenovirus
1961					
17–20 May	1·00	4·55	—	—	—
–24 June	0·65	6·95	0·22	1·20	0·65
–28 July	1·11	7·43	0·61	0·95	0·39
–32 Aug.	0·90	9·15	1·55	0·75	0·55
–36 Sept.	0·94	8·10	0·89	1·34	0·84
–40 Oct.	0·48	7·86	1·30	2·55	0·10
–44 Nov.	1·02	8·32	1·74	2·13	0·29
–48 Dec.	1·47	5·36	1·42	1·47	0·34
–52	1·18	2·96	0·62	1·73	0·56
1962					
1–4 Jan.	0·40	1·34	0·34	0·91	0·23
–8 Feb.	0·28	0·97	0·28	0·77	0·77
Sabin vaccination began					
–12	0·89	1·44	0·28	0·17	0·50
–16 Mar.	3·37	1·36	0·41	0·36	0·71
–20 Apr.	6·63	2·28	0·06	0·18	0·36
–24 May	9·26	2·10	0·28	0·48	1·04
–28 June	9·30	3·08	0·52	0·52	0·39
July					
Total	2·13	5·08	0·77	1·09	0·50

The isolation rates of viruses in 4-weekly periods are shown in Table 5. The rate for polioviruses increased strikingly after the introduction of Sabin vaccination. Coxsackie A and B viruses and echoviruses were more frequently isolated in the summer and autumn than in other seasons. The variations in the isolation rates of adenovirus with season were not statistically significant.

Poliomyelitis vaccination history

The isolation rate of polioviruses from Salk vaccinated children was less than that from unvaccinated children in the period before Sabin vaccination began. After this the relationship was reversed, the isolation rate being higher in Salk

vaccinated than in unvaccinated children (Table 6). The isolation rates of Coxsackie viruses A and B, echoviruses, salmonellae, shigellae and enteropathogenic *Escherichia coli* were unrelated to the history of Salk vaccination. Adenovirus isolations were more frequent in unvaccinated children than in Salk vaccinated children but this was due to the age distribution of adenovirus infections, the isolation rate being highest in children under 1 year when most of them were unvaccinated.

Table 6. *Isolations of poliovirus by type in relation to vaccination history before and after Sabin (trivalent vaccine) vaccination*

Vaccination history of children	Percentage of specimens positive			
	Total	Type 1	Type 2	Type 3
June 1961–February 1962 before Sabin vaccination				
Salk	0·71*	0·39	0·07	0·25
Not vaccinated	1·22*	0·80	0·10	0·32
Total	0·86	0·51	0·08	0·27
March 1962–July 1962 after Sabin vaccination				
Salk	3·71†	1·14	1·14	1·77
Sabin	59·26	27·16	37·04	35·80
Salk and Sabin	66·89	27·15	23·84	47·02
Not vaccinated	2·54†	0·44	1·27	0·93
Total	5·27	1·76	2·03	2·83

Many of the specimens collected after the introduction of Sabin vaccine (February 1962) contained more than one poliovirus type.

* Difference 0·51 % Standard error of difference 0·17.

† Difference 1·17 %. Standard error of difference 0·48.

The isolation rate of poliovirus in Salk vaccinated children varied little between age groups but in unvaccinated children was highest at 1 year and then fell with increase in age (Table 3). Thus, the difference in isolation rates between Salk vaccinated and unvaccinated children was highest in the 1-year age group, that is soon after they had been vaccinated, and it became less with increase in age and increase in the length of time after vaccination. In the 4-year age group there was no significant difference but the figures are too small to permit any conclusions.

Virus isolations by type

Before the introduction of Sabin vaccination Type 1 poliovirus was the most prevalent virus type. It was isolated most frequently in the north and south-west —areas in which outbreaks of Type 1 poliomyelitis occurred during the survey. Type 3 virus, which was less prevalent, was more common in the east midlands than elsewhere in the country (Table 4).

After Sabin vaccination began Type 3 virus became the most prevalent and Type 1 virus the least prevalent (Table 6). This was most evident in children who had received Sabin vaccine after previous Salk vaccination or who had not been vaccinated.

The isolations of Coxsackie A and B viruses, echoviruses and adenoviruses by

types are shown in Table 7. The distribution of each virus type by sex, age, season and type of local authority was similar to that described for all types of the virus together. The geographical distribution by type showed considerable variations, different types being common in different parts of the country at the same time, but there was no evidence of spread of types from one area to another.

Table 7. *Isolations of viruses by type*

Virus type	Number and percentage of isolations							
	Coxsackie A virus		Coxsackie B virus		Echovirus		Adenovirus	
1	1	0·1	8	4·1	21	7·6	37	28·1
2	195	21·8	36	18·4	4	1·4	44	34·4
3	33	3·7	12	6·1	3	1·1	7	5·4
4	184	20·6	91	46·4	—	—	1	0·8
5	123	13·8	41	20·9	1	0·4	20	15·6
6	184	20·6	8	4·1	56	20·1	1	0·8
7	1	0·1	—	—	11	4·0	1	0·8
8	10	1·1	—	—	2	0·7	—	—
9	87	9·7	—	—	46	16·5	1	0·8
10	74	8·3	—	—	—	—	1	0·8
11	—	—	—	—	29	10·4	—	—
12	1	0·1	—	—	4	1·4	—	—
13	—	—	—	—	7	2·5	—	—
14	—	—	—	—	51	18·3	1	0·8
15	—	—	—	—	—	—	3	2·3
16	—	—	—	—	4	1·4	—	—
17	—	—	—	—	3	1·1	1	0·8
18	—	—	—	—	3	1·1	—	—
19	—	—	—	—	8	2·9	—	—
20	—	—	—	—	1	0·4	—	—
21	1	0·1	—	—	6	2·2	9	7·0
22	—	—	—	—	6	2·2	—	—
25	—	—	—	—	7	2·5	—	—
28	—	—	—	—	1	0·4	—	—
Frater	—	—	—	—	4	1·4	—	—
Total all types	894	100	196	100	278	100	128*	100

* In two the type was not recorded.

DISCUSSION

The survey demonstrated that enteroviruses and adenoviruses were often present in the faeces of normal children under 5 years of age, over 8% of all specimens being positive. The prevalence of these viruses varied with sex, age, geographical area, type of local authority and season. The results were similar to those of the previous survey in this country (Report, 1958; Spicer, 1961; Gamble, 1962) and were in accord with those of studies of enterovirus infections in Czechoslovakia (Žáček *et al.* 1962), Hungary (Dömök, Molnár, Jancsó & Dániel, 1962) and the United States (Cole, Bell, Beeman & Huebner, 1951; Honig *et al.* 1956; Melnick, Walton, Isacson & Cardwell, 1957; Gelfand, 1959; Gelfand, Holguin, Marchetti & Feorino, 1963).

Dalldorf & Weigand (1958) reported experiments on monkeys in which paralytic disease followed double infection with attenuated poliovirus and Coxsackie A virus, but neither virus alone produced paralysis. It is possible that a similar summation of the effects of two non-interfering enteroviruses might take place in human beings (Meers, 1965). It is of interest, therefore, that in this survey of healthy children ten double infections with poliovirus and Coxsackie A virus were found. In seven of these the polioviruses were almost certainly wild strains because the specimens were collected before the introduction of Sabin vaccination.

The geographical distribution of the viruses isolated (Table 4) confirms the findings of previous studies that their prevalence varies between areas (Gelfand, 1961; Dömök *et al.* 1962). Interference between poliovirus and Coxsackie B virus was demonstrated by Dalldorf & Albrecht (1955), but in this survey there did not appear to be any relation between the isolation rates of these two viruses either geographically or in time.

All the echovirus types isolated in the survey (Table 7) were isolated also from persons with symptoms in England and Wales in the years 1959–63. Echovirus types 6, 9 and 14 were the most frequently isolated types in the survey, and these types predominated in clinical specimens in 1961 and 1962 (Peckham, 1964). Coxsackie B virus Types 2, 4 and 5 were the most frequently isolated types in the survey and also from clinical specimens (Vernon, 1964). The type distribution of Coxsackie A virus isolations from patients was not the same as in this survey, Type A 9 being found most frequently (Vernon, 1964), probably because few clinical specimens were examined in newborn mice. Adenovirus Types 1, 2 and 5 were the types most often isolated. This was to be expected because these types are commonest in children and usually associated with symptomless infection (Pereira, 1959).

Salk vaccination

Although Fox, Gelfand, LeBlanc & Rowan (1958) found no evidence that Salk vaccination had any effect on virus excretion during a subsequent poliovirus infection, more recent studies have shown that a high serum content of artificially induced antibody diminishes the frequency, amount and duration of excretion (Dick *et al.* 1961; Howe, 1962; Henry *et al.* 1963; Dane, 1964). The results of this survey are in keeping with these findings.

It is probable that Salk vaccination limits the spread of poliovirus in the community by diminishing faecal and pharyngeal excretion of virus on subsequent infection (Marine, Chin & Gravelle, 1962) and thereby protects unvaccinated persons as well as the vaccinated. Epidemiological evidence for this community effect of Salk vaccine was reported by Gard (1961) in Sweden and by Stickle (1964) in the United States. More recently in Sweden, Gard (1964) reported the complete elimination of polioviruses as a result of a systematic campaign in which Salk vaccine was given to a high proportion of the population.

In England and Wales in 1961, 5 years after Salk vaccination began, this community effect was not evident. The incidence of poliomyelitis in 1961 was greater than in the previous year (Report, 1961); furthermore, the results of this survey

show that the prevalence of polioviruses in the community was the same in 1961 as it was 3 years before in the previous survey.

Sabin vaccination

There were two findings of importance to the field study of the safety of Sabin vaccine (Galbraith, 1963). First, the geographical distribution of polioviruses by type before Sabin vaccine was introduced showed that, whereas Type 1 virus was most prevalent in the areas where small epidemics occurred—the north and the south-west—Type 3 virus was most prevalent in other areas. When Sabin vaccination began, therefore, vaccine-associated cases of Type 3 poliomyelitis would by chance be more likely to occur in the non-epidemic areas than in the epidemic areas and the reverse would be true of Type 1 poliomyelitis. Clearly, it would have been a mistake to exclude the epidemic areas from the study and consider only the parts of the country in which vaccine-associated cases of Type 3 poliomyelitis would be expected to be more common. Secondly, Type 3 poliovirus became the most prevalent virus type after the introduction of Sabin vaccine (Table 6). This finding was reported also by Gelfand *et al.* (1963) after mass immunization with trivalent vaccine and it was noted in vaccine trials (Report, 1962; Perkins, Yetts & Gaisford, 1963) that Type 3 virus was excreted more frequently and for longer periods than either Type 1 or Type 2 virus. Thus in cases of paralytic disease following vaccination with trivalent Sabin vaccine a high isolation rate of Type 3 virus would be expected and would not indicate that the disease was caused by this virus.

The isolation rate of poliovirus after Sabin vaccination was introduced was higher in Salk vaccinated children than in unvaccinated children (Table 6). The reason for this is not clear, but it was probably due to intrafamilial spread of vaccine virus. The families that had accepted Salk vaccine for one child, would be likely to accept Sabin vaccine, when it became available, for a younger child; whereas a family that had not accepted Salk vaccine for one child would be unlikely to accept Sabin vaccine for another. Thus it is probable that the Salk vaccinated children were more likely to have been in contact with Sabin vaccinated siblings than the unvaccinated children.

In the study of Gelfand *et al.* (1963) mass immunization with trivalent attenuated vaccine caused a large temporary fall in the prevalence of wild enteroviruses. Sabin vaccine was used for routine immunization only in our survey and its introduction had no striking effect, but the lower isolation rates of Coxsackie A and B viruses and echoviruses in May and June 1962 than in the same months of the previous year may have been due to the vaccine.

SUMMARY

In a survey of enterovirus and adenovirus excretion in normal children in 1961–62, 25,600 faecal specimens were examined, 25,589 of them in cell culture and 17,596 in newborn mice.

Polioviruses were isolated from 156 (0·86 %) specimens before Sabin vaccination was introduced in February 1962 and from 389 (5·27 %) specimens after this.

Coxsackie A viruses were isolated from 894 (5·08%) specimens, Coxsackie B viruses from 196 (0·77%), echoviruses from 278 (1·09%) and adenoviruses from 128 (0·50%).

The isolation rates of these viruses varied with sex, age, geographical area, type of local authority area and season.

The isolation rate of polioviruses before the introduction of Sabin vaccination was the same as in a previous survey carried out in 1957–58, although the rate was lower in Salk vaccinated children than in unvaccinated children. After Sabin vaccination began the isolation rate increased and Type 3 virus replaced Type 1 virus as the most prevalent virus type.

This investigation was undertaken with the assistance of a financial grant to the Public Health Laboratory Service from the Medical Research Council.

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APPENDIX A

The following virologists and bacteriologists took part in the survey: Drs J. A. Boycott, C. M. Patricia Bradstreet, F. A. J. Bridgwater, H. R. Cayton, D. R. Christie, Suzanne Clarke, G. T. Cook, D. G. Davies, J. M. S. Dixon, Lynnette M. Dowsett, A. D. Evans, A. L. Furniss, D. R. Gamble, E. H. Gillespie, M. H. Hambling, L. A. Hatch, R. J. Henderson, H. D. Holt, K. E. A. Hughes, M. H. Hughes, J. E. Jameson, W. H. Jebb, A. C. Jones, W. F. Lane, L. A. Little, G. B. Ludlam, F. O. MacCallum, E. M. Mackay-Scollay, A. D. Macrae, Hélène J. Mair, N. S. Mair, P. G. Mann, B. P. Marmion, E. R. Mitchell, T. D. F. Money, B. Moore, Marguerite S. Pereira, R. Pilsworth, Pauline M. Poole and L. Robertson, Prof. D. T. Robinson, Drs Mary O. Roebuck, W. Ryan, J. A. Rycroft, B. R. Sandiford, A. J. Kingsley Smith, C. E. D. Taylor, Joan Taylor, Prof. Scott Thomson, Drs J. O'H. Tobin, R. L. Vollum, G. Bruce White, J. E. M. Whitehead, Margaret A. Wilson, A. E. Wright.

The following medical officers of health and their staffs co-operated in the survey and undertook the task of collecting faeces specimens from a sample of children in their areas: Drs B. A. Astley-Weston, L. D. Bailey, J. H. Baines,

E. Bebbington, P. M. J. Bobbett, W. G. Booth, J. W. Bowen and W. Bowen-Owen, Prof. D. B. Bradshaw, Drs H. Bryant, H. S. Bury, T. M. Clayton, J. S. Cookson, A. R. Darlow, G. Dison, O. C. Dobson, William Dodd, R. J. Dodds, F. H. M. Dummer, M. L. Dunlop, J. V. Dyer, R. M. Dykes, A. Elliott, J. F. Galloway, W. A. Glen, R. A. Good, I. Gordon, A. R. Graham, H. F. Green, E. Grundy, W. C. Harvey, R. C. Holderness, A. C. Howard, W. D. Hyde, E. D. Irvine, C. Ive, D. J. Jones, R. Arnallt Jones, T. Jones, J. D. Kershaw, S. Leff, Mary Lennox, F. D. M. Livingstone, J. Stevenson Logan, W. D. H. Macfarland, A. C. Mackenzie, J. Maddison, K. N. Mawson, D. F. Morgan, R. B. Morley-Davies, H. Morrison, J. B. Morwood, B. J. L. Moss, J. R. Murdock, L. F. McWilliams, P. J. O'Connell, G. M. O'Donnell, W. S. Parker, D. E. Parry-Pritchard, D. S. Pickup, J. R. Preston, J. L. Rennie, G. M. Reynolds, P. G. Roads, Llywelyn Roberts, H. D. H. Robinson and R. M. Ross, Prof. A. B. Semple, Drs C. L. Sharp, V. D. N. Shaw, E. T. Shennan, D. A. Smyth, L. Spencer Stephens, J. W. Starkey, R. A. Stenhouse, E. W. Caryl Thomas, G. C. K. Thompson, J. F. Warin, D. W. Wauchob, R. C. Webster, P. Westcombe, C. Robertson Wilson, R. C. Wofinden, J. L. M. Wood and A. Yarrow.

Public Health Laboratories and Local Authorities taking part in the survey

Survey area and Registrar-General's region	Laboratory	Local Authority			
		County Borough	Municipal Borough or Urban District		Rural District
North England and North Wales, I II, X, VIII (Wales 2)	Carlisle Chester Conway Leeds Liverpool Preston Sheffield Wakefield	Blackpool Carlisle Chester Leeds Liverpool Sheffield Wakefield	Colne Conway Darwen Fleetwood	Llandudno Lytham St Annes Nelson	—
East Midland, III	Leicester Nottingham	Leicester Nottingham	Beeston and Stapleford	Carlton Loughborough	—
West Midland IX	Birmingham Coventry Hereford Shrewsbury Stafford Worcester	Coventry Dudley Smethwick West Bromwich Wolverhampton Worcester	Droitwich Evesham Hereford Leamington Spa Malvern Nuneaton	Redditch Rugby Shrewsbury Stafford Sutton Coldfield Wednesfield	Droitwich Evesham Pershore Rugby Upton-on-Severn
East Anglia, IV	Bedford Chelmsford Ipswich Luton Norwich Southend	Norwich Southend	Ampthill Bedford Benfleet Biggleswade Brentwood Canvey Island	Chelmsford Clacton Colchester Ilford Kempston Luton Rayleigh	Ampthill Bedford Biggleswade Rochford
London, V	Colindale Epsom	—	Beddington and Wallington Caterham and Warlingham Coulsdon and Purley Ealing Edmonton Enfield Epsom and Ewell Esher Feltham Friern Barnet Harrow Hayes and Harlington	Malden and Coombe Mitcham Ruislip and Northwood Southgate Sunbury-on-Thames Surbiton Twickenham Uxbridge Wembley Willesden Wood Green Yiewsley and West Drayton	—
South-east England, V, VI	Brighton Guildford Maidstone Oxford Portsmouth Winchester	Brighton Oxford Portsmouth	Andover Beckenham Bromley Chatham Eastleigh	Gillingham Horsham Rochester Winchester	Andover Hollingbourn Kingsclere and Whitchurch Maidstone Malling
South-west England and South Wales, VII, VIII (Wales 1)	Bath Bristol Cardiff Exeter Taunton	Bath Bristol Exeter	Barry Gelligaer Nantyglo and Blaina	Rhondda Wellington	—

J. Hyg., Camb. (1965), **63**, 457
Printed in Great Britain

Infections acquired in medical wards

A Report from the PUBLIC HEALTH LABORATORY SERVICE†

(*Received* 12 *April* 1965)

This is an account of an investigation into the incidence of infections acquired by patients in medical wards. Records were collected of 6740 admissions to 13 provincial hospitals between September 1961 and August 1962. Of these, 6282 were to 16 wards in which observations were made on at least 500 patient-weeks of stay, and covered the whole year. The remaining 458 patients were observed in five different wards, all but one of which were included in the investigation for a few weeks only. All 6740 patients were considered in calculations of the incidence of infections acquired in hospital, but only the 6282 patients in the first 16 wards were used in comparisons between wards. All patients admitted to the wards on or after the starting date were included. Transfers from other wards, or re-admissions to wards included in the survey, were not distinguished from other admissions.

DESCRIPTION OF THE WARDS

Table 1 shows the main characters of the sixteen wards that are considered separately in this report. A ward was defined as a section of a hospital in the charge of one day-sister. Thus, an associated pair of wards—one for males and one for females—with different sisters were considered as separate units. There were 13 general medical wards (seven for males, five for females, and one for patients of either sex), one paediatric ward, and two wards for long-stay patients with tuberculosis.

Most were of the traditional open type, with a few side rooms, but some were rather more subdivided. Ward 1 was the only one in which there were no more than four patients in any room. The distance between the bed-centres in the large

† The following took part in the investigation:

Physicians: Dr A. G. V. Aldridge, Dr W. L. Anderson, Dr J. Benn, Dr W. D. Brinton, Dr G. R. Davies, Dr H. R. Davies, Dr A. W. B. Edmunds, Dr P. R. Graves, Dr M. Hamilton, Dr A. C. C. Hughes, Dr D. H. Isaac, Dr R. S. Johnson, Dr A. R. Kelsall, Dr G. A. Kiloh, Dr D. A. F. McGill, Dr I. Martin-Scott, Dr H. K. Meller, Dr D. E. Meredith, Dr H. R. B. Norman, Dr P. M. O'Connor, the late Dr G. D. Owen, Dr E. J. T. Prettejohn, Sir John Richardson, Dr R. T. Rouse, Dr T. L. H. Shore, Dr G. H. Templeman, Dr J. O. Terry, Dr P. G. Todd, Dr A. A. Williams, Dr J. Williams.

Bacteriologists: Dr J. D. Abbott, Dr R. Blowers,* Dr J. A. Boycott,* Dr W. Harris, Dr R. J. Henderson,* Dr M. H. Hughes, Dr R. I. Hutchinson, Dr M. P. Jevons, Dr G. B. Ludlam, Dr B. Moore, Dr H. D. S. Morgan,* Dr D. J. H. Payne, Dr R. Pilsworth, Dr P. M. Poole, Dr H. Schwabacher.

Those marked with an asterisk, together with Dr N. S. Galbraith, Dr M. T. Parker and Dr I. D. G. Richards, constituted the committee which organized the investigation. Dr Blowers acted as secretary and was responsible for the day-to-day organization. The results were analysed by Dr Parker and Dr Richards. Dr Parker was chairman of the committee and prepared the report. Requests for reprints should be sent to him at the Central Public Health Laboratory, Colindale Avenue, London, N.W. 9.

Table 1. *Summary of characters of medical wards*

(The wards bracketed were associated male and female wards in the same hospital. M = male; F = female.)

Ward	Sex of patients	No. of beds: official bed-complement	No. of patients observed	Percentage of beds occupied	Distance between centres of beds (ft.)	Ward subdivision. No. of rooms containing beds							Isolation facilities	Staff (sisters, nurses, auxiliaries and cadets)	
						1	2	3	4	6–12	13–19	21–28		Day	Night
General medical															
1	M+F	32	344	77	*	4	2	—	6	—	—	—	Good	8	2
2	M	37	603	86	7·5	—	2	1	—	2	1	—	Moderate	9	4
3	M	26	306	77	*	2	—	—	2	2	—	—	Poor	11	2
4	F	23	331	90	8	4	—	—	—	—	1	—	Poor	12	1½
5	M	18	222	80	7	1	1	1	—	1	—	—	Moderate	8	1½
6	F	21	236	93	8	1	—	—	—	1	1	—	Moderate	8	1½
7	M	30	533	97	6·3	—	1	2	—	—	—	1	Poor	9	2
8	F	25	484	96	6·3	—	—	1	—	—	—	1	Poor	9	2
9	M	25	528	90	9	1	—	—	—	—	—	1	Poor	9	2
10	M	15	268	93	6	3	—	—	—	1	—	—	Moderate	10	3
11	M	30	704	88	6	2	—	—	—	—	—	1	Poor	9	2
12	F	29	368	90	6	2	—	—	—	—	—	1	Poor	7	3
13	F	38	601	86	9	1	—	—	—	—	1	1	Poor	13	2
Paediatric															
14	M+F	26	588	112†	6	4	4	—	—	—	1	—	Good	7½	2½
Tuberculosis: long-stay															
15	M	28	79	90	12	2	—	—	—	—	2	—	Moderate	12	1
16	F	22	87	92	10	2	—	—	—	2	—	—	Moderate	10	1
Five other wards	—	—	458	—	—	—	—	—	—	—	—	—	—	—	
		Total	6740												

* Distance between centres of beds varied. † Extra beds used.

open wards ranged from 6 to 9 ft., but was 10–12 ft. in the two tuberculosis wards. The proportion of beds occupied throughout the year was 80 % or more in all but two wards, and in the children's ward considerably exceeded the official complement.

Facilities for isolation, and the use made of them, varied widely. In over half of the wards, under 10 % of the beds were in rooms containing two or less beds. In eight of the 13 general medical wards the side rooms were seldom available for use as isolation rooms. In Table 1 we have described the isolation facilities as 'good' in two wards where at least a quarter of the accommodation was in small rooms. There were six others where it was the custom to use the few side-rooms for isolation; these facilities are described as 'moderate'. In the remaining eight wards there were virtually no facilities for isolation.

There was little difference between the general wards in the average length of stay of the patients (2·0–2·9 weeks), and less than 10 % of the patients stayed for longer than 6 weeks. The average stay in the paediatric ward was 2·3 weeks. In the tuberculosis wards, on the other hand, patients stayed on average 15·4 and 11·4 weeks respectively.

At least three-quarters of the patients in each of the general wards were aged 40 years or over. There were, however, considerable differences between wards in the proportion of old people. Two wards (nos. 4 and 8) included more than 30 %, and five others (nos. 3, 6, 11, 12 and 13) between 25 % and 30 % of patients aged 70 years and over, but in wards 1, 2 and 5 the proportion was 15 % or less. The patients in the tuberculosis wards were generally younger; less than 2 % were aged 70 years or more, and 35 % of males and 50 % of females were under 40 years of age. In the paediatric ward 40 % of the patients were less than 1 year old.

Most of the patients in the survey were suffering from one or more major medical illnesses. The commoner diagnoses included cardiovascular disease (coronary artery disease 785, cardiac failure 588, hypertension 296), pneumonia or bronchitis (1048), disease of the nervous system (890), diabetes mellitus (553), and malignant disease (446, including leukaemia).

CLINICAL OBSERVATIONS

A record form was completed for each patient. The bacteriologist visited the ward at least once a week and, together with the physician or his deputy, considered the clinical and bacteriological evidence to decide which patients had acquired an infection in hospital. A list of infections to be noted was printed on the back of the record form (see Table 2). It was made clear to participants that only clinical illness—not simply bacteriological evidence of infection—should be included. Details of antibiotic and corticosteroid treatment (including the name of the drug, the dates on which it was given, and the route), and of all surgical procedures—whether therapeutic or diagnostic (Table 3)—were also recorded.

When a patient was discharged or died, the bacteriologist and the physician jointly completed a section of the form recording the final diagnosis and stated whether any of the specified infections were present on admission or acquired in hospital. They also stated whether the clinical diagnosis of infection acquired in

hospital was supported by bacteriological evidence, and whether in their opinion the infection delayed discharge from hospital or contributed to death.

BACTERIOLOGY

Instructions were given that a nose swab should be taken from each patient on the day of admission, and thereafter weekly. Blood-agar plates were inoculated and examined for *Staphylococcus aureus* (coagulase-positive staphylococci), but enrichment cultures were not made. One colony of *Staph. aureus* from each positive plate was tested for resistance to penicillin, streptomycin, tetracycline, choramphenicol, and erythromycin by the methods in routine use in the various laboratories. All strains of *Staph. aureus* were phage-typed by the method of Blair & Williams (1961) with the 22 phages of the Basic Typing Set (Report, 1963) at routine test dilution (R.T.D.) and at R.T.D. × 1000.

The results of bacteriological investigations relevant to the survey were transferred to the record form.

Table 2. *Infections recorded*

A. Respiratory tract	Tonsillitis Bronchitis Pneumonia
B. Local sepsis	1. Primary infections of skin: pustular lesions (e.g. boils, carbuncles, styes); vesicular or crusted lesions (e.g. impetigo, pemphigus) 2. Skin sepsis secondary to other skin lesions 3. Sepsis following surgical operation, diagnostic procedure or injection
C. Urinary tract	
D. Alimentary tract	Stomatitis Gastro-enteritis
E. Conjunctivitis; otitis media	
F. Other septic lesions	

Table 3. *Surgical procedures recorded*

Surgical operation	Aspiration/injection of pleural cavity
Biopsy	Aspiration/injection of peritoneal cavity
Transfusion by needle	Aspiration/injection of joint
Transfusion by cut-down	Urinary-tract catheterization
Lumbar puncture	Bronchoscopy
Marrow puncture	Other

RESULTS

Infections acquired in medical wards

An infection causing symptoms which began 2 or more days after admission to a ward was considered to have been acquired in it. A total of 345 patients (5·1 %) suffered from one or more infections; 65 patients (1·0 %) suffered from local sepsis of the skin or of wounds, 134 (2·0 %) developed an infection of the lower respiratory tract (acute bronchitis or pneumonia), and 80 (1·2 %) acquired an infection of the urinary tract.

Table 4. *Nature of infections acquired in medical wards: results of bacteriological examination*

(In parentheses: number of times an organism was the only pathogen isolated.)

	No. of infections	Number examined bacteriologically	No pathogens isolated	No. of cultures yielding: *Staph. aureus*	*Str. pyogenes*	Faecal streptococci	Pneumococci	*Haemophilus influenzae*	Coliform bacilli	*Proteus* sp.	*Pseudomonas pyocyanea*	*Clostridium welchii*	*Candida* sp.
Lower respiratory tract infections													
Bronchitis	40	28	6	7 (6)	—	—	6 (5)	4 (3)	5 (2)	2 (1)	1 (0)	—	1 (0)
Pneumonia	95	51	3	26 (19)	—	—	15 (9)	9 (4)	8 (4)	2 (0)	1 (1)	—	—
Local sepsis of skin and wounds													
Primary skin infections: boils, styes	24	23	3	20 (20)	—	—	—	—	—	—	—	—	—
Other primary skin infections	9	8	1	7 (6)	—	—	—	—	—	1 (0)	—	—	—
Secondary skin infections	20	16	0	11 (7)	1 (0)	4 (0)	—	—	3 (1)	5 (2)	2 (0)	—	—
Wound infections	19	12	0	9 (8)	—	1 (0)	—	—	2 (1)	1 (0)	3 (1)	—	—
Urinary tract infections	81	80	1	8 (8)	—	13 (4)	—	—	53 (40)	16 (9)	4 (2)	—	1 (0)
Stomatitis	8	6	0	2 (1)	—	—	—	—	—	—	—	—	5 (4)
Tonsillitis	21	20	10	7 (7)	3 (3)	—	—	—	—	—	—	—	—
Gastro-enteritis	16	13	6	—	—	—	—	—	4 (4)*	—	—	3 (3)†	—
Conjunctivitis	23	16	8	4 (3)	—	—	1 (1)	4 (3)	—	—	—	—	—
Otitis media	13	4	0	3 (3)	—	—	—	—	—	—	1 (1)	—	—
Other septic lesions	15	10	0	6 (6)	—	—	—	—	1 (1)	—	1 (1)	2 (2)	—
Total	384	287	38	110 (94)	4 (3)	18 (4)	22 (15)	17 (10)	76 (53)	27 (12)	13 (6)	5 (5)	7 (4)

* Infantile gastro-enteritis associated with *Esch. coli* O 119.

† Food-poisoning.

There were 384 separate infections with symptoms (5·7 per 100 patients admitted). Table 4 shows the numbers in each of the main clinical groups and summarizes the bacteriological findings in them. One-third of the infections were of the lower respiratory tract (pneumonia 25 %, acute bronchitis 10 %), one-fifth were local infections of the skin or of wounds, and one-fifth were urinary tract infections (Fig. 1 A).

Information about the bacteriology of illnesses diagnosed as *acute bronchitis and pneumonia* was obtained by examination of sputum, occasionally supplemented by cultures made *post mortem*, in 79 (59 %) of the 135 patients on whom these diagnoses were made. When more than one specimen of sputum was examined, an organism was considered as a possible cause of the disease only if it was

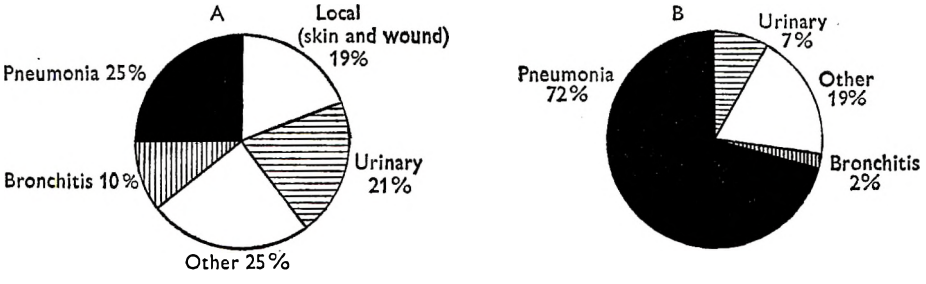

Fig. 1. Main clinical groups of infections acquired in medical wards.
A, All infections; B, infections contributing to death.

present in the first specimen taken after the onset of illness. A possible pathogen was isolated from 22 of 28 cases of acute bronchitis; *Staph. aureus* was obtained from seven, and was the only pathogen found in six; the rest yielded pneumococci, *Haemophilus influenzae*, and other organisms in various combinations. All but three of 51 cases of pneumonia yielded a possible pathogen. The commonest organism was *Staph. aureus*, which was present in 26 (51 %), and was the only significant organism in 19 (37 %). Pneumococci were isolated from 29 %, *H. influenzae* from 18 %, and various Gram-negative bacilli from 22 %.

Twenty-four of the 33 primary skin infections were boils or styes, and the remainder comprised two whitlows, five septic skin rashes, one case of impetigo and another of superficial cellulitis. *Staph. aureus* was isolated from 27 of the 31 that were cultured. Secondary skin infections occurred in 18 bed-sores, one rodent ulcer, and one case of minor trauma. Half of those cultured were infected with *Staph. aureus* only, and the rest with a mixture of organisms. The wound infections were somewhat different from those usually seen in surgical wards, for only seven of them followed a major surgical operation; the rest included four infections of tracheostomy wounds, two of biopsy wounds, and five which followed injections or similar procedures. *Staph. aureus* was isolated from 9 of the 12 that were swabbed.

All but one of the 81 urinary tract infections were investigated bacteriologically. A single organism was responsible for the following percentages of cases: coliform bacilli 50, *Proteus* spp. 11, *Staph. aureus* 10, faecal streptococci 5, *Pseudomonas pyocyanea* 2; and 20 % were mixed infections with similar organisms.

The 16 cases of gastro-enteritis included a small outbreak of food-poisoning due to *Clostridium welchii* affecting three patients in a general medical ward, and four infections of infants with *Escherichia coli* O 119 in the paediatric ward. There were no salmonella or shigella infections. Gastroenteritis in two patients was attributed to antibiotic therapy, but the illnesses were mild.

The 15 remaining infections described as 'other septic lesions' were various, but included several of the more severe illnesses. There were three bacterial infections of the parotid gland, two dental abscesses, a deep abscess of the buttock, an abscess of the foot, and a case of acute sinusitis. One patient had had purulent meningitis before admission, but cultures of the cerebrospinal fluid were sterile; in the ward he developed acute staphylococcal pericarditis. Two other patients, one with aplastic anaemia and the other with congestive heart failure, were found to have staphylococcal septicaemia without localized suppuration. There were two fatal infections due to *Cl. welchii*; one was a terminal gas-gangrene in a case of aplastic anaemia and the other—in a patient with rheumatoid arthritis under treatment with steroids—an acute ileal ulceration with *Cl. welchii* septicaemia. One generalized infection with *Ps. pyocyanea* in a patient with renal failure was acquired after intravenous and intra-arterial catheterization. Another probable generalized bacterial infection—in a patient with aplastic anaemia—was diagnosed histologically on post-mortem material, and may have resulted from a blood transfusion.

Consequences of infection acquired in hospital

In all, 345 patients acquired an infection in hospital, and 94 of them died before they could be discharged. In the opinion of the investigators, infection contributed to death in 59 (17 %) of these patients, and may have had some influence on the outcome of another 12. The discharge from hospital of 41 of the 251 survivors (16 %) was considered to have been delayed by infection, but the length of the delay could not be estimated accurately.

Table 5. *Acquired infections which contributed to death*

	Age (years)				All ages
	< 1	1–49	50–69	> 70	
All patients	234	2337	2811	1358	6740
Total deaths	15	78	307	331	731
Infections contributing to death					
Total	4	6	23	26	59
Pneumonia	4	6	12	21	43*
Bronchitis	0	0	1	0	1
Urinary tract infection	0	0	1	4	5*
Other	0	0	9	2	11
Fatal staphylococcal infections (pneumonia in parentheses)	1 (1)	0	8 (4)	3 (3)	12 (8)

* One patient had pneumonia and urinary-tract infection.

Infections were thought to have played a part in 8 % of the 731 deaths in the wards, and pneumonia was the infection most often implicated (Table 5; Fig. 1 B).

The 60 infections concerned in the death of 59 patients included 43 illnesses described as 'pneumonia', 'bronchopneumonia', 'terminal pneumonia' or 'hypostatic pneumonia', and one case of acute bronchitis; but the diagnosis was, in most cases, clinical and unsupported by bacteriological evidence. No examination of the sputum of 28 of the patients was made at the relevant time. *Staph. aureus* was isolated from the sputum, or from the lung *post mortem* in eight of the 16 cases examined. Nearly half of the patients with fatal pneumonia or bronchitis acquired in the wards were aged 70 years or more, and most of them were already suffering from a serious illness; 13 had cerebral thrombosis or haemorrhage, 11 had congestive heart-failure, coronary thrombosis, or hypertension, five had generalized carcinomatosis, and three were diabetics. All four of the babies dying of pneumonia had a severe congenital defect or birth injury.

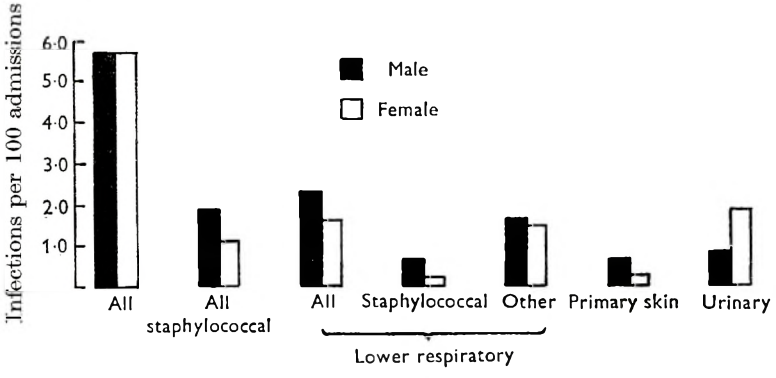

Fig. 2. Incidence of infection acquired by males and females in medical wards.

The remainder of the fatal infections included five of the urinary tract, three cases of extensive sepsis in bedsores, and six generalized bacterial infections (two due to *Staph. aureus*, two to *Cl. welchii*, one to *Ps. pyocyanea*, and one to an unknown organism).

Age and sex

Table 6 shows the incidence of infections acquired by male and female patients of various ages, expressed as the number of infections per 100 patients admitted. The rate for all infections was identical in the two sexes. The highest incidence was in the first year of life and the next highest in patients over the age of 70. The lowest was between the ages of 10 and 30, and the infection rate rose slowly through middle life.

When the individual types of infection are considered, differences between the sexes appear (Fig. 2). There was an excess of males over females in all staphylococcal infections, in infections of the lower respiratory tract, and in primary skin infections. The incidence of staphylococcal infections of the lower respiratory tract was 0·67 for males and 0·19 for females, but the remainder of the respiratory tract infections were evenly distributed between the sexes.

The excess of primary skin infections is particularly noticeable in young and middle-aged males. Nine of the 33 infections occurred in one ward for long-stay tuberculosis patients, but there was no evidence that they were caused by a parti-

Table 6. *Incidence of acquired infection by age and sex*

(M = male; F = female.)

Age (years)	No. of admissions M	No. of admissions F	Incidence per 100 patients admitted: All infections M	All infections F	All staphylococcal infections M	All staphylococcal infections F	Infections of: Lower respiratory tract M	Lower respiratory tract F	Urinary tract M	Urinary tract F	Skin (primary) M	Skin (primary) F	Skin (secondary and wounds) M	Skin (secondary and wounds) F
< 1	146	88	13·0	8·0	4·8	0	0·7	3·4	0	0	0·7	0	3·4	0
1–	200	123	4·5	4·1	0	1·6	0·5	0	0	0·8	0	0	0·5	0
10–	177	117	1·1	2·6	1·1	0·9	0	1·7	0	0	0·6	0	0	0
20–	236	188	3·8	2·7	1·3	0·5	0	1·1	0	0	1·7	0	0	0·5
30–	290	207	4·8	3·9	2·4	0·5	0·7	1·0	0·7	1·4	2·1	0	0·3	0·5
40–	498	301	4·4	2·0	1·8	0·7	1·6	0	0·4	0·3	3·0	0·3	0·4	0·7
50–	901	400	4·7	5·5	1·4	0·8	2·3	1·0	0·7	2·3	0·6	0	0·1	0·5
60–	992	518	5·6	7·1	2·0	2·3	2·6	1·9	1·0	2·7	0·2	1·0	0·8	0·6
70–	725	633	8·7	8·7	2·8	1·1	4·8	2·8	1·7	3·3	0·4	0	0·7	1·1
Total	4165	2575	5·7	5·7	1·9	1·1	2·3	1·6	0·8	1·9	0·65	0·23	0·55	0·62

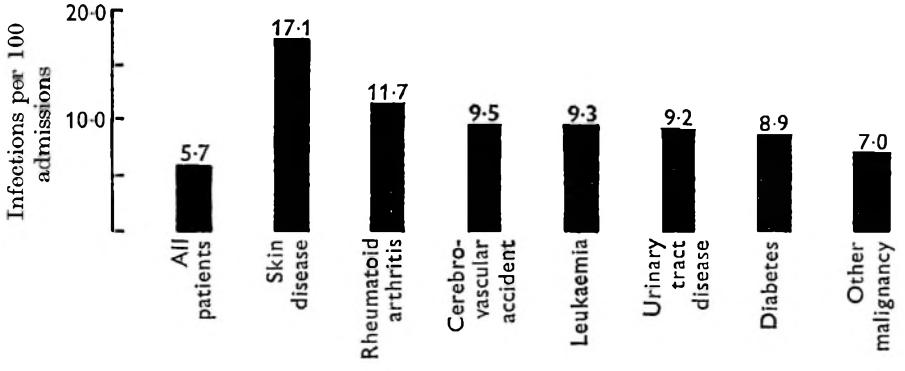

Fig. 3. Relation between primary disease and liability to infection.

cular strain of staphylococcus, and a comparable number of female patients of about the same age in an associated ward did not suffer from any primary skin infections. Apart from the cases in the tuberculosis ward the numbers of primary skin infections among males and females under 60 years of age were respectively 13 and 1.

Urinary tract infections, on the other hand, were much more common in females than in males, and were almost entirely confined to patients over 30 years of age.

Relation between primary disease and liability to infection

The incidence of acquired infections per 100 admissions by patients with particular diseases was compared with the rate for all patients (Table 7; Fig. 3). Those suffering from more than one disease were included in the total for each disease.

The incidence of infection in patients suffering from coronary artery disease, cardiac failure, hypertension, pneumonia, bronchitis, or peptic ulceration, showed little deviation from the average for all patients, apart from a slight excess of urinary tract infections and pulmonary infections in females with congestive heart failure.

Table 7. *Infections acquired by patients admitted to hospital with certain diseases*

Diagnosis on admission	No. of admissions		Incidence per 100 patients admitted: All infections		Staphylococcal infections		Lower respiratory tract		Urinary tract infections	
	M	F	M	F	M	F	M	F	M	F
Coronary artery disease	607	178	5·8	7·3	2·1	0	3·5	1·7	0·5	2·2
Cardiac failure	359	229	5·3	9·2	1·1	0·4	1·7	3·9	1·9	3·9
Hypertension	180	116	6·1	3·4	0·6	0	4·4	0·9	0·6	0·9
Pneumonia	340	166	5·9	4·8	2·4	1·8	2·6	1·2	0·9	0·6
Bronchitis (acute and chronic)	374	168	7·0	6·0	1·9	1·2	3·5	3·0	0·5	1·8
Peptic ulcer	234	83	6·8	0	3·0	0	3·0	0	1·3	0
Urinary tract disease (including infections)	187	237	13·9	5·5	4·3	1·3	2·1	1·3	6·4	1·3
Cerebrovascular accident	242	168	10·3	8·3	2·1	0·6	5·4	1·2	1·7	5·4
Other nervous system diseases	280	160	7·9	6·3	3·6	1·2	1·4	3·1	1·4	1·3
Skin diseases	66	45	16·7	17·8	10·6	6·7	0	4·4	3·0	8·9
Malignancy (including leukaemia)	357	109	6·4	10·0	3·1	2·8	3·1	4·6	1·1	0·9
Diabetes	295	258	8·1	9·7	3·0	1·5	2·4	1·6	2·7	5·4
Rheumatoid arthritis	44	93	15·9	9·7	2·3	4·3	11·4	0	0	4·3
All patients	4165	2575	5·7	5·7	1·9	1·1	2·3	1·6	0·8	1·9

Male patients admitted with a disease of the urinary tract were liable to develop acute urinary infection while in hospital. Nine of the 12 patients in this class had retention of urine due to prostatic hypertrophy or carcinoma, and two more had gross anatomical abnormalities of the kidney or ureter; nine had been treated with a self-retaining catheter and one had had a cystoscopy. The two patients who had not been catheterized had carcinoma of the prostate.

Patients suffering from cerebral thrombosis or haemorrhage often acquired an infection in hospital. The excess of pulmonary infections, however, consisted mainly of cases of 'terminal' or 'hypostatic' pneumonia, and little bacteriological information was available about them. The incidence of urinary tract infections in these patients was also high. There was, however, a difference between the sexes in the apparent role of catheterization in causing these infections; all five of the males had recently been catheterized, three with a self-retaining catheter, but only one of the eight females had been catheterized.

Patients admitted with a disease of the skin ran a very high risk of acquiring infection (17·1 infections per 100 admissions), especially one due to *Staph. aureus* (9·0 per 100 admissions). Few of them, however, were strictly dermatological patients; most also suffered from other serious medical diseases. For this reason, and because there were only 111 such patients, no conclusions could be drawn about the hazards associated with particular skin diseases.

The rate of infection in patients with malignant disease was not as high as might be expected, but a wide variety of diseases, at varying stages of their evolution, were included. Thus, there were only eight infections among 194 cases of bronchial carcinoma, but many of the patients had been admitted to the ward for diagnosis only. There were five infections among 54 cases of leukaemia (9·3 per 100 admissions).

There was not a great excess of staphylococcal infections in diabetic patients, but half of all the staphylococcal urinary tract infections occurring among 6740 patients were in the 553 diabetics. Urinary tract infections, both in males and females, were very common. There was an association with catheterization in males but not in females; the infection followed catheterization in five of eight males, but in only four of 14 females.

Infection was common in rheumatoid arthritis (11·7 per 100 admissions), but the small number of patients at risk made further analysis difficult. There were three fatal infections among 137 patients, two due to *Staph. aureus* (one septicaemia and one pneumonia) and one to *Cl. welchii*.

Table 8. *Infections following certain surgical and diagnostic procedures*

(In parentheses: infections per 100 procedures.)

	No. of procedures			No. of infections acquired			
	Male	Female	Both sexes	Infection	Male	Female	Both sexes
Surgical operation	76	43	119	Sepsis	7	4	11 (9·2)
				Bronchitis	1	1	2 } (9·2)
				Pneumonia	4	5	9 }
Biopsy	40	24	64	Sepsis	1	1	2 (3·1)
Bronchoscopy	42	12	54	Tonsillitis	3	0	3 }
				Bronchitis	1	0	1 } (9·3)
				Pneumonia	1	0	1 }
Transfussion (needle)	186	122	308	Sepsis	1	2	3 (1·0)
Transfusion (cut-down)	51	22	73	Sepsis	2	0	2 (2·7)
Catheterization (ordinary)	48	61	109	Urinary tract	3 (6·3)	4 (6·6)	7 (6·4)
Catheterization (indwelling)	40	31	71	Urinary tract	15 (37·5)	5 (16·1)	20 (28·2)

Infections following certain surgical and diagnostic procedures

Procedures were included in this study (Table 8) only if they were performed more than 5 days before the patient was discharged from hospital, and—except for catheterization—were considered relevant only to symptoms occurring within

7 days of them. There were 119 surgical operations, of which 11 were followed by wound sepsis and 11 by chest infection, giving a wound-infection rate of 9·2 % and a total post-operative infection-rate of 18·5 %. Also, two of 64 biopsies were followed by local sepsis, both in patients with skin disease.

Two transfusions by needle were followed by 'red arm', one with evidence of thrombophlebitis, and one other may have been responsible for a generalized bacterial infection. One transfusion by cannula caused local staphylococcal wound infection and another resulted in fatal generalized infection with *Ps. pyocyanea*. Unfortunately there was some confusion in the definition of the procedures to be described as transfusions: at twelve centres all intravenous therapy by drip was included, but at one centre only intravenous administration of blood was considered to be a transfusion. Thus, the sepsis-rates of 1·0 % for simple transfusion and 2·7 % for cannulation may be slightly too high.

Table 8 also shows the relation between urinary tract infection and previous catheterization. Simple catheterization was performed 109 times, and seven infections followed (6·4 %). Self-retaining catheters were inserted 71 times, with 20 associated infections (28·2 %). The incidence of urinary infection in all uncatheterized patients of both sexes was only 0·9 % of all admissions. Catheterization seemed relatively more important as a cause of urinary infection in males than in females. Eighteen of 32 males, but only nine of 49 females who acquired a urinary tract infection had previously been catheterized.

No infection followed 304 lumbar punctures, 66 marrow punctures, and 105 aspirations from or injections into the pleural or peritoneal cavity or into a joint. There was one example of local sepsis after acupuncture and two intramuscular abscesses at the site of penicillin injections.

The administration of antibiotics and steroids

One-third of the patients received an antibiotic by injection or by mouth whilst in hospital, and 7 % were treated with a steroid or with ACTH. Table 9 shows the percentage of patients in each ward who received an antibiotic or a corticosteroid, and also the percentage treated with penicillin, tetracycline, chloramphenicol and streptomycin. The amount of antibiotic and steroid therapy varied considerably from ward to ward, but the most striking differences were in the use of penicillin, tetracycline and chloramphenicol in different wards. The percentages receiving other antibiotics were as follows: erythromycin 1 %, methicillin 0·7 %, neomycin 0·6 %, ampicillin 0·4 %, novobiocin 0·2 %, and polymyxin, bacitracin, framycetin, and kanamycin all less than 0·1 %.

We tried to relate the administration of antibiotics and of corticosteroids to the subsequent appearance of infection. Courses of either given after the onset of symptoms were not considered. There was a significant excess of all infections, and of staphylococcal infections, both among those receiving antibiotics and those receiving corticosteroids. It appeared to be common practice, however, to give prophylactic antibiotics to old and sick patients who had a greater risk of contracting an infection. Similarly, the patients receiving corticosteroids included many who were already very susceptible to infection, particularly those suffering

from leukaemia and rheumatoid arthritis. It seems unwise, therefore, to draw firm conclusions about the relation of antibiotic and corticosteroid therapy to acquired infection from the information obtained in this survey.

Table 9. *Percentage of patients receiving antibiotics and corticosteroids*

(Excluding topical applications.)

Ward no.	Percentage receiving: Any antibiotic	Steroids or ACTH	Penicillin	Strepto-mycin	Tetra-cycline	Chloram-phenicol
1	18	4	14	0·6	10	0·9
2	36	5	4	2	32	6
3	38	3	26	4	16	1·0
4	37	6	25	3	16	0·9
5	14	7	7	3	6	0·9
6	22	6	12	3	9	0·8
7	24	6	6	0·8	4	23
8	25	10	7	1	5	13
9	22	2	15	7	8	1·5
10	40	5	22	2	22	1·5
11	30	6	17	6	12	8
12	34	8	21	10	12	8
13	36	8	14	4	11	16
14	35	5	22	2	3	9
15	62	4	15	48	15	5
16	64	10	8	54	14	—
All patients	34	7	15	5	13	8

Incidence of infection in individual wards

Table 10 shows the number of infections per 100 weeks of ward exposure in the 16 wards where the numbers of observations were sufficient for separate analysis. The total infection rate exceeded 2·5/100 patient-weeks in seven of the general medical wards; in six of them, and only one other, the incidence of lower respiratory tract infection was greater than 1·0 per 100 patient-weeks. In general, these seven wards were also those with the highest death-rates attributable to infection, and those in which the greatest proportion of deaths were thought to have been due to infection, but there were several exceptions.

The incidence of staphylococcal infection in individual wards did not correspond closely with the total infection-rate, or with the incidence of infections contributing to death. It exceeded 0·8 per 100 patient-weeks in two of the six wards with high death-rates from acquired infection (> 0·5/100 patient-weeks), but also in two of the seven with lower death-rates. There was only one ward (no. 11) in which a high incidence of fatal respiratory tract infection was attributable, at least in part, to an excess of staphylococcal pneumonia. This was the only ward in which there was evidence of an 'epidemic' of staphylococcal infection associated with a single strain of *Staph. aureus*.

There were, however, great differences between wards in the frequency with

which bacteriological examination was performed on patients suspected of suffering from respiratory tract infections, and this may account for part of the difference between the recorded incidences of staphylococcal pneumonia. In wards 3 and 4, for example, none of the 20 patients said to have acquired pneumonia or bronchitis had a sputum examination after the onset of the illness. There were, however, six wards in three hospitals where the sputa of at least half of the patients with these infections were examined bacteriologically. The number of observations was rather small (Table 11), but there appeared to be differences between wards in the frequency with which *Staph. aureus* was present in sputum from cases of bronchitis and pneumonia acquired in hospital.

Table 10. *Number of infections, and number of infections contributing to death, in each ward*

(In parentheses: infections per 100 patient-weeks of exposure.)

	Infections							
			Staphylococcal		Lower respiratory			Death: % attributed to infection
Ward no.	Total	Contributing to death	All	Primary skin	All	Staphylococcal	Urinary tract	
1	6 (0·6)	0	2 (0·2)	1	1 (0·1)	1	3	0
2	20 (1·5)	1 (0·1)	7 (0·5)	1	10 (0·7)	5	5	1
3	30 (3·4)	11 (1·2)	4 (0·5)	1	11 (1·2)	0	3	22
4	15 (1·7)	6 (0·7)	0	0	9 (1·0)	0	3	15
5	7 (1·5)	0	4 (0·8)	1	2 (0·4)	2	0	0
6	19 (3·2)	1 (0·2)	3 (0·5)	0	7 (1·2)	3	7	4
7	34 (2·7)	7 (0·6)	6 (0·5)	0	17 (1·4)	2	6	10
8	33 (2·8)	5 (0·4)	12 (1·0)	4	5 (0·4)	0	11	11
9	21 (1·8)	0	13 (1·1)	5	6 (0·5)	2	4	0
10	19 (3·4)	5 (0·9)	3 (0·5)	2	8 (1·4)	1	5	20
11	52 (3·7)	9 (0·6)	23 (1·6)	1	30 (2·1)	12	9	9
12	28 (3·7)	5 (0·7)	7 (0·9)	2	10 (1·3)	1	6	11
13	32 (2·3)	4 (0·3)	2 (0·1)	0	7 (0·5)	1	18	5
14	40 (2·9)	5 (0·4)	9 (0·7)	0	5 (0·4)	1	1	25
15	18 (1·5)	0	10 (0·8)	8	4 (0·3)	1	0	0
16	4 (0·4)	0	1 (0·1)	0	0	0	0	0
Other	6 (0·6)	0	4 (0·4)	1	3 (0·3)	1	0	0

Table 11. *Bacteriological findings in cases of pneumonia and bronchitis in six wards*

Ward no.	Total no.	No. examined bacteriologically	*Staph. aureus* isolated	Other possible pathogens isolated	No possible pathogen isolated
5	2	2	2	0	0
6	7	7	3	4	0
7	17	14	2	9	3
8	5	3	0	2	1
11	30	25	12	10	3
12	10	5	1	3	1

There was no apparent correspondence between the number of ward subdivisions, the quality of the facilities for isolation (Table 1), the proportion of elderly patients (p. 459), or the antibiotic policy (Table 9), and the risk of acquiring infection. The most that can be said is that the incidence of all infections was low in the general medical wards nos. 1, 2 and 5, where there were good or moderately good facilities for isolation, and few patients over 70 years of age; but the record was not much worse in several wards with less favourable physical conditions. In the long-stay tuberculosis wards, with good bed-spacing, a rather stable population, and a small proportion of elderly people, there was little evidence of serious infection.

Epidemiology of acquired staphylococcal infection

The investigators recorded 110 staphylococcal infections in 101 patients (1·5 per 100 admissions). *Staph. aureus*, alone or in association with other organisms, was implicated in the deaths of 12 patients, eight with staphylococcal pneumonia, two with terminal bacteraemia, one with pericarditis and one with a septic bedsore.

One hundred and twelve distinct cultures of *Staph. aureus* were isolated from the 110 lesions, and all but two of them were tested for sensitivity to antibiotics. Twenty-one % were sensitive to all antibiotics, 14% were resistant only to penicillin, and 65% were resistant to other antibiotics. The percentages resistant to each were as follows: penicillin 77, streptomycin 50, tetracycline 55, chloramphenicol 3, erythromycin 7.

Table 12. *Phage-typing patterns of* Staphylococcus aureus *strains responsible for infections of* 99 *patients*

	Pattern	Total cases	Endemic infection*	Sporadic infection
Phage group I	'80/81'†	27	23	4
	52A/79	4	0	4
	29 and 29 patterns	4	2	2
	Other	6	0	6
Phage group II		3	0	3
Phage group III	7/47/53/54/75	13	13	0
	83A	9	2	7
	6/7/53/75	2	2	0
	42E	3	2	1
	Other	14	0	14
Miscellaneous		5	0	5
Not typable		9	0	9
Total		99	44	55

* Two or more infections due to the same strain of *Staph. aureus* in one ward.
† Includes 52/52A/80/81 and similar patterns, if resistant to penicillin.

A nasal swab was taken within one day of admission to the ward from 95 patients who later developed 102 of the lesions. The organisms responsible for 21 of these lesions were already present in the nose when the patient was admitted, but nine of them were resistant to antibiotics other than penicillin, suggesting that

they had been derived directly or indirectly from a hospital. Admission swabs were examined from 26 patients who later suffered from primary staphylococcal skin infections, and 13 of them (50%) contained the infecting organism. Few patients who later developed other staphylococcal lesions were carrying the infecting organism at the time of admission. The proportions were as follows: for wound infections 1/8, for pneumonia and bronchitis 3/22, for urinary tract infection 0/8, and for other lesions 4/20.

Table 13. *Incidents of endemic staphylococcal infection and nasal acquisition of* Staphylococcus aureus

(Column 2. *Endemic infections.* Strains of *Staph. aureus* which gave rise to two or more clinical infections in each ward, and the number of infections caused. Column 3. *Nasal acquisition.* Staphylococcal strains responsible for 10% or more of nasal acquisitions in each ward. (In italic: phage-typing patterns at R.T.D. × 1000).)

Ward no.	No. of patients with clinical infections (1)	Endemic infections (2)	Nasal acquisition (3)
1	2	—	'80/81': 22%
2	7	'80/81': 4	'80/81': 25%
3	4	—	83A: 34%
4	0	—	'80/81': 14% 83A: 14%
5	3	'80/81': 2	*7/47/53/54/75*: 25%
6	3	—	*7/47/53/54/75*: 17%
7	6	83A: 2	'80/81': 19%
8	12	'80/81': 3 42E: 2	'80/81': 25%
9	10	'80/81': 6	'80/81': 17%
10	3	'80/81': 2	'80/81': 29% 53/54: 12%
11	21	7/47/53/54/75/77: 13 '80/81': 2	7/47/53/54/75/77: 16% 83A: 14%
12	6	'80/81': 2	'80/81': 29%
13	2	—	*6/7/42E/47/53/54/75/77*: 10%
14	9	'80/81': 2 6/7/53/75: 2	'80/81': 10%
15	7	29/52: 2	29/52: 65%
16	1	—	7/54: 18%

Phage-typing was carried out on the staphylococci from 99 lesions. The results are summarized in Table 12, in which distinction is made between endemic and sporadic infections. An endemic infection was one due to a strain of *Staph. aureus* which caused two or more clinical illnesses in the same ward during the year. Only 44 of the 99 infections belonged to incidents of endemic infection. The remaining 55 were sporadic infections due to organisms responsible for only one illness in a particular ward. Half of the staphylococci responsible for endemic infections were members of type '80/81', i.e. were lysed by phage 80 or 81, and sometimes also by phages 52 and 52A, but by no others. Endemic infection with '80/81' staphylo-

cocci existed in eight of the 16 wards, but the incidents were all small. There was one relatively large group of 13 infections due to a strain with the phage-typing pattern 7/47/53/54/75, and four pairs each due to a different organism. Thus, the 13 distinct endemic incidents were due to organisms with only six different phage-typing patterns, and only 14 of the 55 sporadic infections were due to organisms with these patterns (see also columns 1 and 2 of Table 13).

We tried to compare the part played by certain strains of *Staph. aureus* in causing endemic infections with their ability to colonize the nose of patients. Column 1 of Table 13 shows the number of patients in each ward who acquired staphylococcal infection, and column 2 shows the incidents of endemic infection. Column 3 shows nasal acquisitions of certain staphylococcal strains as percentages of all acquisitions in the same ward. They were obtained from the regular weekly swabbing of 4100 of the 6740 patients in the survey (61 %). Only those strains are shown which accounted for 10 % or more of all nasal acquisitions.

When a staphylococcal strain caused a series of infections in a ward it was usually also being disseminated actively among the patients, but not always. Thus eight of the 13 strains responsible for two or more lesions also constituted 10 % or more of the staphylococci acquired in the nose by patients in the same ward. Three more strains were ones acquired by smaller percentages of patients, but two incidents (in wards 8 and 14) were due to organisms apparently not acquired at all by nasal carriers in the ward. In the first there was strong evidence that one patient introduced the organism into the ward and infected the other, but in the second there was no apparent connexion between the two patients.

On the other hand, there were many instances in which a staphylococcal strain that was actively disseminated among the patients did not cause a group of infections; 11 'carrier endemics', which resulted in over 10 % of all the acquisitions in a ward, were due to strains which did not cause endemic sepsis (see Table 13). Nor can the absence of endemic clinical infection by these strains be attributed to low susceptibility of the patients exposed to them, because in several instances other staphylococcal strains were causing infections at the same time (e.g. wards 5, 10, 11).

DISCUSSION

This investigation was planned as a counterpart to the Public Health Laboratory Service survey of the incidence of surgical wound-infection (Report, 1960), but it proved much more difficult to obtain a clear-cut picture of sepsis in medical wards than in surgical wards. Most patients undergoing an operation have a good prognosis, and the course of their convalescence can be predicted fairly accurately at the time of operation. Patients in medical wards, on the other hand, often suffer from diseases which are themselves fatal and which run a less predictable course. It proved impossible, therefore, to compare the expected date of discharge with the actual date, and so to assess the part played by infection in increasing the length of stay in hospital as was done in the surgical-ward survey.

The information collected was the sum of the individual clinical judgements of many physicians. They were asked to seek bacteriological confirmation of their

diagnoses whenever this seemed necessary and practicable; but the extent to which they made use of the laboratory, and the weight they attached to the bacteriological evidence varied widely.

Infections of the lower respiratory tract accounted for one-third of the acquired infections, and for nearly three-quarters of those which contributed to death. As well as being the most important single group of infections, this was the most difficult one to assess. Most of the patients affected were elderly, and nearly all were suffering from some other serious disease. Inspection of the individual case-sheets suggested that the criteria used for diagnosis were far from uniform. Great difficulties were experienced in interpreting the bacteriological findings in pneumonia. In some hospitals little significance was attached to the results of sputum culture, and the examination was seldom if ever carried out. In others, sputum was examined regularly, and the organisms isolated from patients with clinical signs of pneumonia were considered to be the cause of the disease. The number of patients on whom bacteriological investigations were made *post mortem* was too small for separate analysis, and it is doubtful whether regular post-mortem bacteriology would have contributed much additional information. It is well established (Norris & Pappenheimer, 1905; Smillie & Duerschner, 1947; Finland & Jones, 1956; Mitchell, Dunn, Lees & Hedges, 1961; Rantasalo & Hjelt, 1963; Emson, 1964) that pneumococci, *Staph. aureus* and other potential pathogens are often found in the lungs in the absence of naked-eye signs of inflammation, particularly in patients who have been in hospital for some days (Järvinen, Kahanpää, Rantasalo & Fortelius, 1961). It is therefore necessary to be very cautious in interpreting results of cultures from lung swabs and from sputum of hospital patients (see Weiss & Flippin, 1963).

In the present investigation, just over half of the patients who acquired pneumonia in hospital were examined bacteriologically. An organism to which the infection could conceivably have been attributed was isolated from 48 of the 51 patients. *Staph. aureus* was isolated from half of them, and was the only significant pathogen found in over one-third; it was also isolated from a similar proportion of the fatal cases of pneumonia, though only one-third were examined bacteriologically. If we assume that *Staph. aureus* was equally common in the cases that were not examined bacteriologically, and that all patients with clinical pneumonia and staphylococci in the sputum were suffering from staphylococcal pneumonia, we should conclude that the disease had an incidence of 48 in 6740 admissions (0·7 %) and was concerned in 22 out of 731 deaths (3 %). This is almost certainly an over-estimate.

In a concurrent autopsy survey of staphylococcal infection among medical and surgical patients (Report, in the Press), the conclusion was reached that staphylococcal pneumonia was a contributory cause in 6·4 % of 470 deaths, but between one-third and two-thirds of these infections were probably terminal events in patients who could not have expected long survival.

Local sepsis of the skin and of wounds was a relatively unimportant problem in the medical wards. Primary skin infections due to *Staph. aureus* were mild and sporadic. Half of them were due to organisms present in the patient's nose on

admission to the ward. Secondary skin infections were mainly in bedsores; three were believed to have contributed to the death of patients. Wound sepsis followed 9·2 % of surgical operations—a proportion comparable with that found in surgical wards (Report, 1960).

Urinary tract infection was over twice as common in females as in males. There was a significant association between catheterization and urinary tract infection in both sexes, but catheterization was relatively of greater importance as a cause of infection in males than in females. Thus, over half of the infections in males, but less than one-fifth of those in females, occurred in those who had been catheterized in hospital. The relatively high incidence of urinary infection in uncatheterized females is difficult to explain. Probably some of the infections thought to have been acquired in hospital were recrudescences of latent pyelonephritis.

The proportion of patients who acquired a staphylococcal infection (1·5 %) did not differ greatly from that reported in other surveys. Minchew & Cluff (1961) found evidence of staphylococcal infection other than wound sepsis in 1·1 % of patients in a general hospital (see also Farrer & Macleod, 1960; Frohman *et al.* 1964). Galbraith & Bailey (1964) recorded an incidence of 1·3 % in patients in four medical wards in one hospital. A considerably higher infection-rate (3·8 %) was, however, observed by Shooter, Girling, Matthias & Williams (1960) in a ward containing many patients with malignant and blood diseases.

An estimate of the mortality from staphylococcal disease based on the twelve fatal infections that were diagnosed bacteriologically gives a rate of 0·18 per 100 admissions, but this is an uncertain figure because of the difficulties in the diagnosis of staphylococcal pneumonia. If we assume that staphylococcal infection was as common among all fatal cases of pneumonia as it was in those examined bacteriologically, the mortality-rate for all hospital-acquired staphylococcal diseases would be 0·38 per 100 admissions. Rogers & Bennett (1958) estimated that between 0·35 and 0·95 % of medical patients in the New York Hospital acquire 'staphylococcal infections which pose a threat to life'.

The results of this survey do not indicate, as is often suggested, that staphylococcal and other serious infections are rife and are spreading uncontrolled in the wards of our hospitals; but they do show that a few patients in medical wards contract infections and suffer consequences more serious than the conditions for which they entered hospital.

The possible ways of preventing these infections are even less clear than for those occurring in surgical units, because precautions against infections of the type we studied cannot be applied—as for a surgical wound—to a defined body-site during a fairly short period of high susceptibility. Moreover, our findings do not offer great hope of control by isolating known infective patients in side-wards. Thus, though most of the staphylococcal sepsis was due to 'hospital strains' 55 % of it was due to strains which caused only one lesion in the ward during the year, so isolation of that patient would not have prevented another infection.

Many patients acquired 'hospital staphylococci' in the nose whilst in hospital, and the strains causing lesions were generally those prevalent in the noses of patients in the same ward. But there was little correspondence between the rate

of nasal colonization by a particular strain and the amount of sepsis it caused, so the value of isolating patients known to acquire it—even if this were practicable—remains doubtful. We incline to the view of Williams *et al.* (1962) that attempts to do this are unlikely to control staphylococcal disease, and that wards should therefore be built to isolate as many patients as possible from each other.

However, the increased susceptibility to infection of patients in certain age, sex, and disease categories suggests that there would be some advantage for them in protective isolation or segregation.

SUMMARY

A co-operative study was made of the incidence of infection acquired by patients in medical wards. Records were collected of 6740 admissions to 21 wards in 13 hospitals.

There were 384 clinical infections (5·7 per 100 patients admitted); 135 of them (35 %) were infections of the lower respiratory tract, 72 (19 %) were septic skin lesions or infected wounds, and 81 (21 %) were urinary tract infections.

Infection was believed to have contributed to the death of 59 patients—17 % of those infected or 8 % of those dying in hospital. Nearly three-quarters of the deaths were attributed to 'pneumonia'.

Acquired infections were most common and most severe at the extremes of age. There was an excess of males over females in staphylococcal infections and in infections of the lower respiratory tract, and of females over males in urinary tract infections.

The incidence of infection was above average in patients suffering from malignant disease, diabetes, rheumatoid arthritis, cerebral thrombosis and haemorrhage, and from diseases of the urinary tract and of the skin.

There were 110 acquired infections with *Staph. aureus* and 12 deaths were attributed to them. Over half of these infections were due to staphylococcal strains which caused only one clinical infection in a ward in the course of a year.

Pneumonia was difficult to diagnose in severely ill or moribund patients, and its clinical significance was hard to assess. It was not possible to obtain a reliable estimate of the part played by bacterial infection in its causation.

The committee are grateful to the many nurses and laboratory technicians who assisted in the investigation and to Miss B. J. Kinsley for statistical advice.

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Printed in Great Britain

Serological epidemiological studies with influenza A viruses

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(*Received* 3 *May* 1965)

The first studies of the age distribution of antibodies in man against human and swine influenza viruses were made in Britain in 1935 (Andrewes, Laidlaw & Smith, 1935) and in the U.S.A. in 1936 (Francis & Magill, 1936; Shope, 1936). In 1953 a large-scale survey of such antibodies was reported by Francis, Davenport & Hennessy (1953), using sera from Michigan pooled by age group. The results of these tests showed that antibodies against serologically different viruses were present in different amounts in persons of different ages. Each family of influenza viruses thus gave a characteristic antibody distribution by age, and antibodies were not found in pooled sera from persons born after the last recorded isolation of a particular virus family. As a result of these findings Davenport, Hennessy & Francis (1953) formulated the hypothesis that childhood infection by a particular serological family of influenza A viruses imprints upon the antibody-forming mechanism a pattern which is recalled later by antigenic stimuli from serologically different influenza viruses. With swine influenza virus, antibodies were found in high titres in sera from persons over 30 years of age in 1953. Similar results were obtained with sera from Sheffield collected in 1954 (Davenport, Hennessy, Stuart-Harris & Francis 1955), and the findings were thought to support the view suggested by Laidlaw (1935) that though swine influenza virus had never been recovered from human cases of influenza, it or a virus sharing its major antigen was responsible for the 1918 pandemic of influenza.

However, Isaacs (1957), using Nigerian sera, and Masurel & Mulder (1962), with sera from the Netherlands, both reported that they had found low titres of swine virus antibody in individuals under the age of 30 and that the titres rose gradually from the first year of life to a plateau in those of 35 years of age or over. The alternative explanation formulated to explain the existence of low levels of antibody was that swine virus antibodies in human sera might be formed as a result of repeated infections by human influenza viruses sharing minor antigens with swine influenza virus. Such an hypothesis failed to explain the fact that children of 12 or over in Britain and the U.S.A. showed good amounts of swine antibody in 1935, whereas there was a lack of antibody under 12 in 1935 and up to the age of 30 in 1953. However, the early work was done with neutralization tests on individual sera and the later studies were with the haemagglutination-inhibition test on pooled sera.

It was decided that a repetition of the tests with swine influenza virus using individual sera newly collected from many persons of different ages would be helpful in assessing these two hypotheses. Sera collected between 1961 and 1964, 7 or

more years after the first study in Sheffield, were tested by haemagglutination-inhibition and in some instances by tissue culture neutralization methods and the results are here reported. Representative strains of the influenza A, A1 and A2 virus families were used and tests were made with two viruses of equine influenza recovered from horses and two strains of duck influenza virus.

MATERIALS AND METHODS

Sera

The sera from individuals aged 6 months to 60 years were collected in 1961 and 1962 from patients in the Children's Hospital, Sheffield (6 months to 15 years), and the City General Hospital (5–60 years of age). Serum collections made before inoculations of poliovirus vaccine were available from school-children aged 12–16 years, from a teacher's training college (17–19 years) and from employees of the West Riding County Council (18–45 years). Altogether, 524 sera were collected and these were approximately evenly distributed by sex and age.

Sera were also available from forty male employees at an engineering works who volunteered to give blood in 1952 and again in 1963. Their ages in 1952 were between 29 and 52 years.

In 1964 a separate collection of sera was made from aged persons (60–90 years) resident in Fir Vale Infirmary or patients in the Royal Hospital, Sheffield. One hundred and twenty-two sera were collected from fifty-six males and sixty-six females approximately equally distributed in the various decades.

All sera were stored in sealed screw-capped vials at $-20°$ C.

Viruses

A/Swine (Shope Sw. 15, 1930), A/PR 8 (1934), A1/FM 1 (1947) and A 2/Singapore/1/57 were stock strains adapted to growth in the allantoic cavity. A/Equine/Prague/56, A/Equine/Miami/63, A/Anatum/Prague/56 and A/Anatum/England/62 were kindly provided by Dr H. G. Pereira, World Influenza Centre, Mill Hill, London. Pools of virus were made by allantoic inoculation of 10-day embryonated eggs with 10^{-3} or 10^{-4} dilutions of virus. After incubation at 35° C. for 48 hr. the allantoic fluids were harvested, pooled and stored in sealed ampoules at $-70°$ C.

Haemagglutination-inhibition (HI) tests

These were carried out in 'Perspex' trays by a standard technique (World Health Organization, 1953) using 8 haemagglutinating units of virus (50% end-point). Before testing, all sera were treated to remove non-specific inhibitors by incubating overnight at 37° C. with 5 vol. of cholera filtrate (N.V. Philips Roxane) and heating at 56° C. for 1 hr. HI titres were read from the pattern of haemagglutination and expressed in terms of the initial serum dilutions. Ferret antisera prepared against A/Swine, A/PR 8, A1/FM1 and A2/Singapore/1/57 were used as standards. No cross-reactions were detected between these strains using potent ferret antisera. Comparisons of HI titres in paired sera from 1952 and 1963 were always made in a single test with identical reagents.

Neutralization tests (A/Equine/Miami/63)

Equal volumes of inactivated sera diluted 1/5 and virus dilution (100 TCID 50/0·1 ml.) were incubated together at 20° C. for 30 min. before inoculation into two to four rhesus monkey kidney tissue cultures (0·2 ml./tube). Cultures were incubated at 36·5° C. for 3 days and virus growth was then detected by haemadsorption with fowl erythrocytes (Vogel & Shelokov, 1957). Sera showing neutralization at 1/5 were titrated in twofold serial dilutions to determine the end-points. All tissue cultures were maintained in medium 199.

RESULTS

Antibodies in sera from persons of 1–60 years of age

The age distribution of antibodies to the four families of influenza virus A2, A1, A and swine are shown in the accompanying figures (Figs. 1–4). In each figure the solid line represents the proportion of sera at the stated ages in which the haemagglutination-inhibition (HI) test indicated the presence of antibody at a titre of 1 in 9 or more (initial dilution). The geometric mean titres (dotted line) were also calculated from the reciprocals of the haemagglutination-inhibition titres and sera negative at 1 in 6 (the lowest dilution tested) were given the arbitrary rating of 3. The curves for percentages of positive sera and geometric mean titres were closely parallel for each virus. Antibody to A2 virus (Fig. 1) was present in more than 20% of the sera at each age but the highest mean titres occurred at 6–11 years of age. With increasing age the titres fell and reached their lowest levels in persons aged 36–50 again rising, however, to a second peak at 51–60 years of age. With the A1 virus (Fig. 2), antibody was not found in sera from children aged 8 or less but from 9 onwards increasing amounts were detected. Peak titres were reached at 12–20 years of age with a second peak at ages 50–60 and low levels in those aged 31–40. With the A/PR8 virus (Fig. 3), sera from persons aged 15 or less contained no antibody, peak titres occurred at ages 21–30 and a second minor peak occurred at ages 41–50.

These results confirmed the absence of antibodies to A and A1 viruses in sera from those born more recently than 1946 and 1953 respectively. As the last recorded A and A1 epidemics in England occurred respectively in 1943 and 1956 a reasonable agreement exists between the disappearance of a particular virus family from the community and absence of antibody from children's sera. Secondly, the peak titres to A and A1 viruses occurred in those aged 5–10 years more than the youngest persons in whom antibodies were detected. This suggests that the maximum antibody response occurs in the 5–15 age group during the prevalence of a particular virus family as indeed was found with the A2 virus (Fig. 1). This general age distribution of influenza antibodies thus supports the doctrine formulated by Davenport *et al.* in 1953.

Figure 4 shows the distribution of antibodies to swine virus. These were not found in children aged 11 or less in 1961. Negative or a low percentage of positive results (2–8%) were found between 12 and 30 years of age. After 30 years of age

Fig. 1

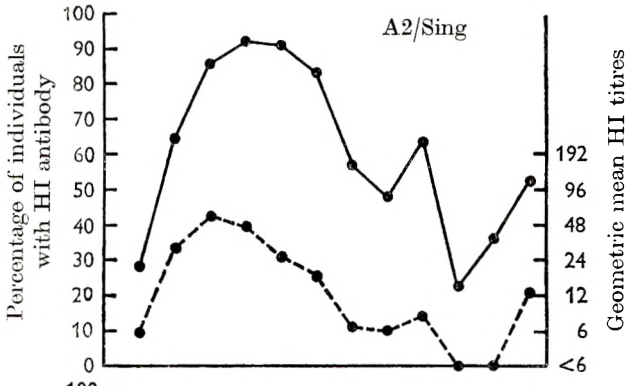


Fig. 2

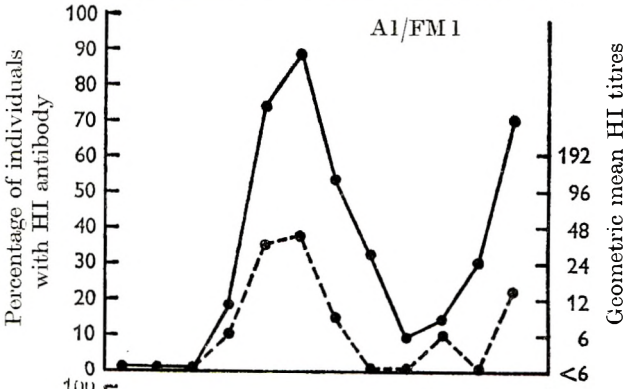


Fig. 3

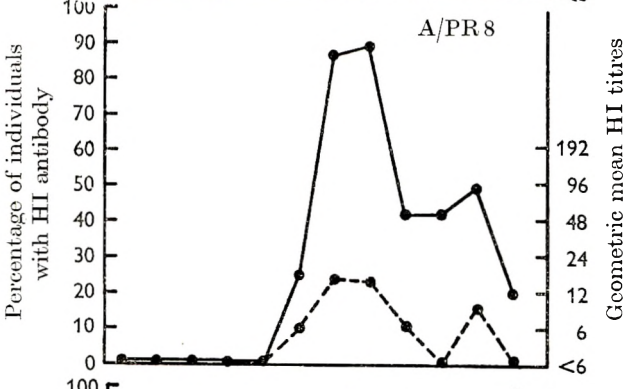
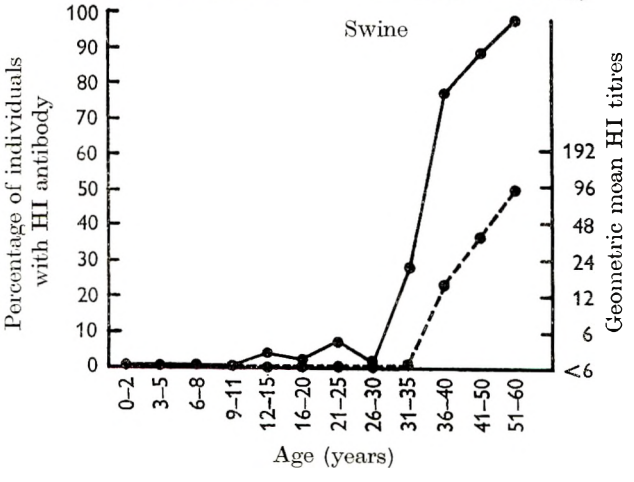


Fig. 4



Figs. 1–4. For legend, see foot of facing page.

the proportion of sera with antibody rose rapidly and peak titres were found in the age group 51–60 years where approximately 100 % of sera contained antibody. These results must be compared with those obtained using pooled sera in Sheffield in 1954 in which no antibody was detected in persons aged 31 or less. It seems probable that tests with individual sera might at that time have revealed antibody in younger persons but it is unlikely that a moderate percentage of positive sera would have been missed. There was, therefore, no obvious change in the age distribution curve for swine antibodies in 1961 compared with that in 1954, whereas the curves for both A and A 1 viruses appeared to have shifted to the right in this period of time. A possible reason for this difference between swine and A and A 1 viruses is considered below.

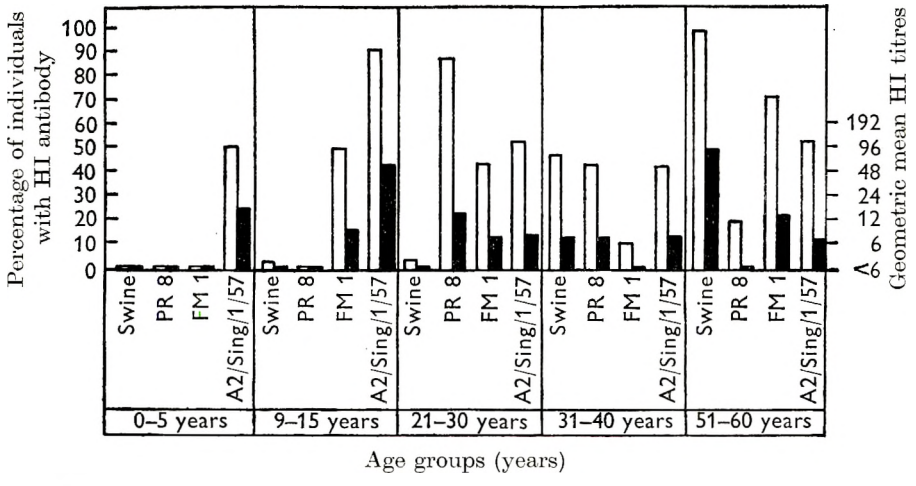


Fig. 5. Summary of the distribution of HI antibodies to swine, A, A 1 and A 2 viruses in various age groups of the population in 1961–62. Open columns = percentage of individuals with HI antibodies. Solid columns = geometric mean HI titres.

Figure 5 shows, in summarized form, the geometric mean titres and proportion of positive sera in selected age groups with each of the four viruses. It is seen that, apart from the swine virus, the findings with the various serological families fit in with the theory that a particular cohort of persons acquires antibodies in childhood to the virus circulating in the community at that time. These antibodies persist even though the particular virus family is replaced by a serologically different strain. A recall of the childhood antibodies formed by infection with swine virus might result from minor shared antigens possessed by the various human viruses. The existence of antibodies to swine influenza virus has been explained by the view that this virus or one sharing its major antigen was the cause

Figs. 1–4. Proportion of individuals with HI antibody and geometric mean HI titres to various strains of influenza A virus in serum specimens collected in 1961–62. Continuous lines = percentage of individuals with HI antibodies; broken lines = geometric mean HI titres. Fig. 1. A2/Singapore/1/57 virus. Fig. 2. FM 1 virus. Fig. 3. PR 8 virus. Fig. 4. Swine virus.

of the 1918 pandemic of influenza. The fact that the curve of swine virus antibodies did not alter much between 1954 and 1961 and that a low percentage of positive sera was detected in young adults in 1961 demanded further inquiry.

Changes in influenza antibodies between 1952 *and* 1963

Paired sera collected in 1952 and again in 1963 from forty adult males living near Sheffield and who were aged 40–63 in 1963 were tested against each of the four viruses used above. Table 1 shows the mean HI antibody titres for the men in

Table 1. *Comparison of mean HI antibody titres in* 1952 *and* 1963 *in the same individuals*

Ages	No. and year of serum specimen	Geometric mean HI titres A2/Sing/1/57	FM 1	PR 8	A/Swine
29–38	15 1952	< 3	5·2	8·3	22
40–49	15 1963	9·1	5·4	10	54
39–48	19 1952	< 3	17	3·4	120
50–59	19 1963	10	16	4·3	158
49–52	6 1952	< 3	8·7	3	69
60–63	6 1963	59·8	16·6	4·6	151
All ages	40 1952	< 3	10	4·6	55
	40 1963	13	10	5·9	100

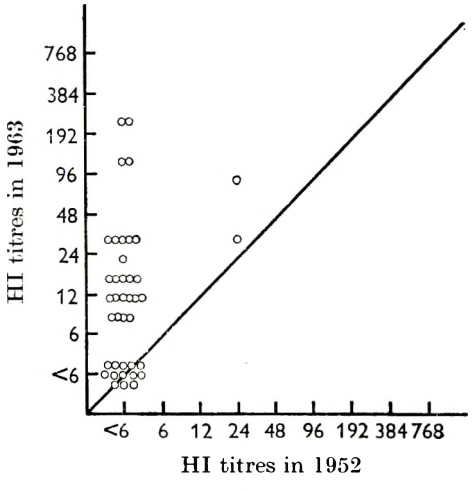


Fig. 6

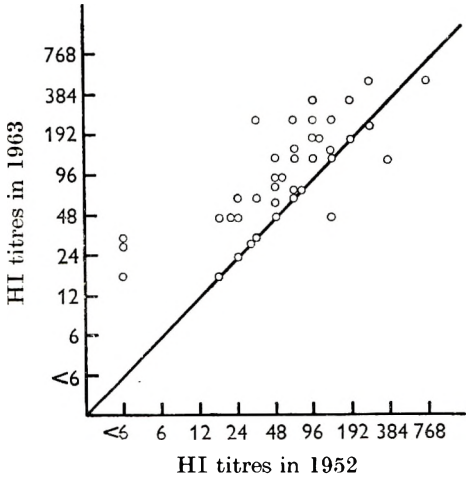


Fig. 7

Figs. 6–7. Changes in HI antibody titres to A2/Singapore/1/57 (Fig. 6) and swine virus (Fig. 7) in the same forty individuals over an 11-year period (1952–63).

various age groups and Figs. 6 and 7 show the actual titres obtained with A 2 and swine virus in individual sera in the two years. Two sera collected in 1952 from persons then aged 34 and 43 contained antibody to A 2 virus and the rest showed no inhibition at a dilution of 1/6. The subsequent acquisition of A 2 virus antibody by twenty-five more of the forty persons was doubtless due to the circulation of this

virus in the epidemics of 1957, 1959 and 1961. Probably some of the thirteen persons whose sera were negative to A 2 virus in 1963 may have had A 2 antibody at some time before 1963 but subsequently lost it. With A 1 virus which caused outbreaks of influenza in the years 1953/54 and 1955/56, some of the forty persons first sampled in 1952 had gained antibody by 1963 but others had lost such antibody. Only in those aged 60–63 in 1963 was there a rise in the mean titres to A 1 virus compared with the titres in 1952 and the means of the other persons were unchanged. With A virus a slight change in mean titres occurred and there were both gains and losses of antibodies in different persons. The overall effect was slight. However, the results with swine virus shown in Table 1 and Fig. 7 were different from those with either the A or A 1 viruses. There was in fact a net gain in mean titres of antibodies to swine virus in persons of all ages and only two individuals underwent a twofold decrease in titre of antibodies between 1952 and 1963. Three persons who had no swine virus antibody in 1952 had developed this by 1963. These changes furnished evidence that the several waves of influenza virus infection to which these forty adults were exposed in the 11-year period between the collections of sera had reinforced their previous antibodies to swine virus. No similar effect was apparent with the A (PR 8) virus so that it seemed probable that the swine virus shares some antigenic grouping either with the A 2 or the A 1 virus family. If this is so, then the lack of shift in swine virus antibodies in the population referred to above (Fig. 4) is explained by the recruitment of antibodies probably by the action of shared antigens. At the same time the lack of decrease in the mean titres to both A and A 1 viruses between 1952 and 1963 in the group of forty persons just described is evidence of the remarkable stability of antibodies to former influenza viruses in the adult population as a whole. It seems that the hypothesis of antigenic recall of antibodies acquired in childhood formulated by Davenport *et al.* (1953) is supported by these findings, but that evidence also exists in favour of the view that minor antigens shared by serologically different virus families also influence the antibody levels found in the population.

Antibodies to equine influenza viruses in sera from aged persons

While the above work was in progress, it was learnt from Prof. Davenport that antibodies to the 1963 but not to the 1956 strains of equine influenza virus had been found in a proportion of sera from aged persons in Michigan. A new collection of sera from persons in Sheffield 60–90 years of age was, therefore, made in 1964 and HI tests were performed with the same swine and human viruses as before and also with the Prague (1956) and Miami (1963) strains of equine influenza virus and two strains of duck influenza virus (A/Anatum/Prague/57 and A/Anatum/England/62).

In the sera from aged persons and also 300 serum specimens from individuals aged 11–55 years no HI antibodies were detected with A/Equine/Prague/56 virus or the two duck strains of influenza virus. Using the A/Equine/Miami/63 virus, which is serologically distinct from A/Equine/Prague/56, antibodies were detected in the HI test in 12–14 % of sera from persons over 70 years of age. The titres were

low and ranged from 1/6 to 1/48. No HI antibodies were found to this virus in 31 sera from persons aged 60–69, nor were any detected in 300 sera from persons aged 11–55 years. To confirm the serological independence of A/Equine/Miami/63 HI tests were carried out using immune ferret antisera with high homologous titres to A/Swine, A/PR 8, A 1/FM 1 and A 2/Singapore/1/57. No cross-reactions with A/Equine/Miami/63 were detected. The antibody patterns to the human and swine influenza viruses with the same sera were similar in those containing equine virus antibodies and in those without such antibodies.

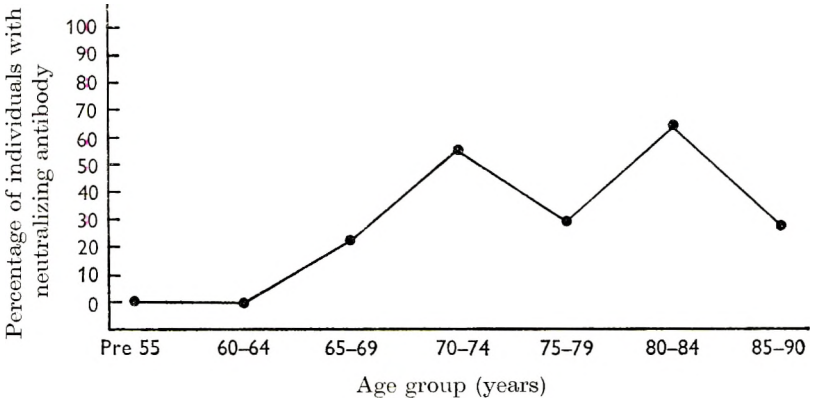


Fig. 8. Proportion of sera from aged people with neutralizing antibody to A/Equine/Miami/63 virus at titres of 1 in 5 or greater.

In order to confirm that the inhibition of the 1963 equine virus by sera from aged persons was due to the presence of antibody, neutralization tests were performed in rhesus monkey kidney tissue culture. All sera in which HI antibody was detected contained neutralizing antibody to A/Equine/Miami/63. The distribution of neutralizing antibody with age (Fig. 8) was generally similar to that of HI antibody but 22 % of those aged 65–69 showed antibody at 1/5 or greater. Neutralizing antibody was not detected in 175 sera from individuals aged 11–55 years but was found in a higher percentage of those over 70 than had been detected by HI test. The tissue culture neutralization technique thus seemed to be a more sensitive method of detecting antibody than the HI test.

Finally, Fig. 9 shows the age distribution of antibody to the human, swine and Miami 1963 strains of influenza A virus in the sera from aged persons. The proportion of positive sera and the geometric mean titres to swine, A, A 1 and A 2 viruses of sera from persons aged 60–69 were similar to those of sera from persons aged 51–60. Over the age of 70, however, sera were less often positive with swine virus and the titres declined in amount, whereas with the other viruses the sera remained much the same as in those under 70. The reason for this decrease in antibody to swine virus could not at first be explained. Separation of the results in women from those in men showed, however, a remarkable difference (Table 2). Over the age of 80, 91 % of male sera contained swine virus antibodies but only 27 % of female sera gave positive inhibition of swine virus. A similar trend was detected in males and females between 70 and 80 years of age (88 % of males and

60 % of females had antibody). No such sex difference was found in sera from persons under 60 years of age, nor did sera from males and females over 60 give different percentage results with other viruses.

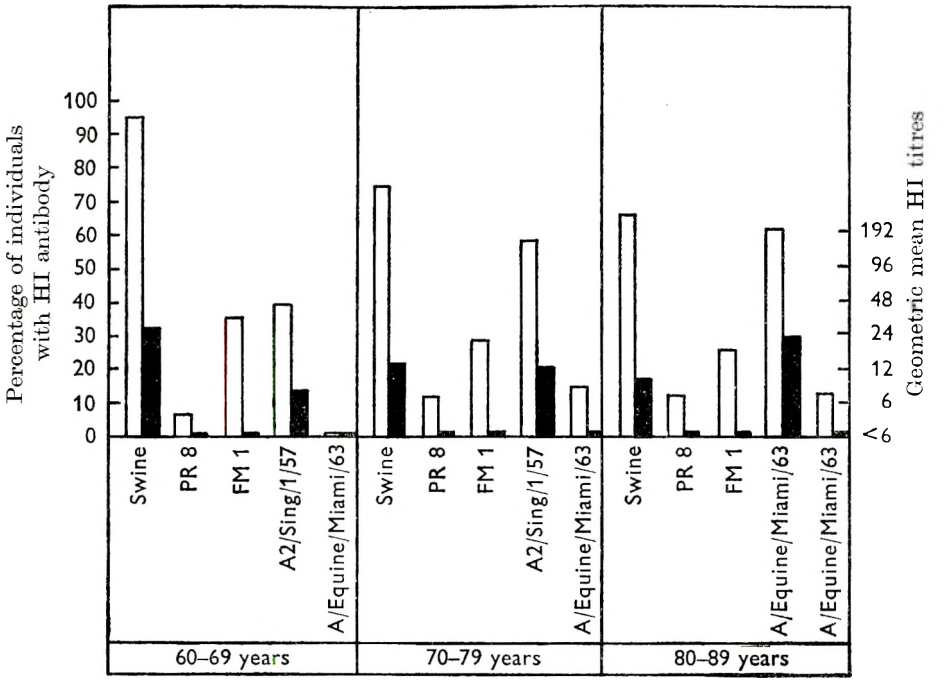


Fig. 9. Distribution of HI antibodies to various influenza A viruses in serum specimens collected in 1964 from aged persons. Open columns = percentage of individuals with HI antibodies. Solid columns = geometric mean HI titres.

Table 2. *Comparison of the proportion of aged males and females with antibody to swine virus in* 1964

Age range (yr.)	Total no. of sera tested	Nos. of sera with HI antibody to swine virus at titres of 1/9 or greater		Percentage of males with antibody	Percentage of females with antibody	Overall percentage positive sera
		Males	Females			
40–59	63	28/29*	32/34	96·6	94·2	95·3
60–69	32	15/15	15/17	100	88·2	93·8
70–79	42	15/17	15/25	88·2	60	71·4
80–89	44	20/22	6/22	90·9	27·3	59·1

* Numerator = number of positive sera; denominator = number of sera tested.

DISCUSSION

The general hypothesis that serum antibodies to influenza viruses not only give information concerning infection by viruses currently circulating in the community but also indicate the antigenic nature of the viruses of former epidemics is not new. Shope's findings (1936) of swine virus antibodies in a high

proportion of sera from young and older adults but not from children led him to argue that this virus or one of similar antigenic composition had been widely prevalent in the recent past. He agreed therefore with Laidlaw (1935) that the swine influenza virus probably represented a surviving form of the human pandemic virus of 1918. All the recent evidence from previous studies and from those reported above are concordant with this hypothesis. The view that the small percentage of adults now under 30 years of age possessing swine virus antibodies represents those in whom antigens of human viruses have produced a heterologous response to swine virus, does not afford an adequate explanation for the existence of a high percentage of adults over 30 with such antibody at the present time. The recent work of Drescher *et al.* (1962*a*, *b*) using the photometric serological technique which permits the separation of specific from cross-reacting antibody is of interest in this connexion. Application of the technique to sera from persons of different ages by Davenport *et al.* (1964) has shown that the swine virus antibody in adults over 40 years of age is specific in its reaction, whereas that from younger persons has the character of cross-reacting antibody. The finding of a shift in age distribution of swine virus antibodies between 1935 and 1953 both in the U.S.A. and in Britain (Davenport *et al.* 1953; Davenport *et al.* 1955) is therefore concordant with the opinion that such antibodies are related to the 1918 epidemic. The lack of any further change in age distribution of swine virus antibodies between 1954 and 1961 in Sheffield may well be due to a cross-relationship between the antigens of swine and A 2 human influenza virus. Such a relationship was suggested by Masurel & Mulder (1962) and evidence in favour of it was obtained by Harboe (1963) in sera from ferrets subjected to repeated infection by various influenza viruses.

The work reported above has thrown no further light on the possible relationship between the A 2 influenza virus and that causing the 1890 epidemic. Mulder & Masurel (1958) were the first to suggest that the A 2 virus was antigenically related to the virus of the 1890 pandemic and their evidence for this was the discovery that in sera collected during the period just before the 1957 A 2 epidemic, antibody to this virus was detected in a small percentage of persons in the Netherlands over the age of 70. Davenport & Hennessy (1958) obtained similar findings in the U.S.A. and so did Davoli & Corsi (1957) in Italy. Yet even Mulder & Masurel (1958) showed that persons immunized with inactivated A 1 vaccine in the pre-Asian era sometimes developed antibody to A 2 virus in the post-vaccination phase. Similar results were obtained in Sheffield (Clarke, Heath, Sutton & Stuart-Harris, 1958), but only occasional instances were found of pre-epidemic A 2 antibody in unvaccinated persons and there was no tendency for concentration of positive specimens in the small number of old persons who were examined. Nevertheless, the results obtained with pre-epidemic 1957 sera by Davenport *et al.* (1964) using the Drescher technique clearly show that the antibody to A 2 virus found in sera before the occurrence of the epidemic was specific in its reaction. It is difficult to escape the conclusion that the A 2 virus antigen had been experienced antigenically by the population before 1957 and possibly in 1890.

The results with the 1963 equine influenza virus are similarly suggestive that this virus is antigenically related to that causing human infection more than 65

years ago. Our own findings confirm those of Davenport (personal communication) and show that antibody capable of neutralizing the virus is certainly present in a proportion of English persons over 65 years of age. Some of the positive sera were sent to Dr Drescher in Berlin who has informed us that the sera react in a specific manner to his test. There is no epidemiological evidence suggesting that equine influenza is a disease at all related to human infection and the serological findings therefore remain unexplained. Davenport *et al.* (1964) use the term 'serological archaeology' for the type of work which led to this discovery and doubtless there is much more yet to be discovered about the viruses of former human influenza epidemics if the appropriate antigens could be located. Certainly it would be unwise to speculate concerning the exact time-relationships between past virus infections and epidemics. It should not be forgotten that aged persons are survivors from a much larger number of persons of their particular cohort. Differences due to chance will almost certainly occur in antibody distributions in the aged therefore. Such a chance selection of survivors may account for the sex difference found in swine virus antibodies in aged Sheffield residents. More work with sera from persons living in other areas is required before deductions are possible.

SUMMARY

Determinations were made of the age distribution of antibody to swine virus and representatives of the various families of human influenza A virus in 1961–62 collections of human sera and paired sera from forty individuals taken in 1952 and 1963:

(*a*) The existence of cohorts of the population, each with a dominant antibody type related to strains of virus first encountered in childhood, was confirmed.

(*b*) The basic epidemiological pattern was similar to that previously detected in 1954. However, it seemed that antibody to swine virus had been reinforced but not antibody to A and A 1 strains.

(*c*) Neutralizing and HI antibodies to A/Equine/Miami/63 virus were detected only in the sera of older people (65 years or over) collected in 1964. No antibodies were found to A/Equine/Prague/56 or two duck viruses.

(*d*) Relatively constant levels of antibody to A, A 1 and A 2 viruses were present in sera from aged persons but antibody to swine virus diminished with age. This could be attributed to a lack of swine antibody in the older females.

The authors are indebted to Mrs Dorothy Edey, A.I.M.L.T., for excellent technical assistance. We also wish to thank Dr C. W. Potter of the University of Sheffield, Dr P. Howard of the Royal Hospital, Sheffield, Dr S. S. Missan of the City General Hospital, Sheffield, and Dr R. W. Elliott, County Medical Officer for the West Riding of Yorkshire for their kind help in obtaining sera.

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Note added in proof:

Since this communication was submitted for publication workers in the U.S.A. have reported finding HI antibody to a 1963 strain of equine influenza A virus in sera collected in 1958 from older persons in Michigan (Minuse, E., McQueen, J. L., Davenport, F. M. & Francis, T., Jr. (1965). Studies of antibodies to 1956 and 1963 Equine influenza viruses in horses and man. *J. Immunol.* **94**, 563). The peak incidence of this antibody was in persons born between 1880–1890.

Further, Dr Masurel (personal communication) wishes us to state that he has detected similar antibodies in the sera of older persons in Leiden.

J. Hyg., Camb. (1965), **63**, 491
Printed in Great Britain

An evaluation of Rappaport's magnesium chloride/malachite green medium in the routine examination of faeces

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(*Received* 6 *May* 1965)

Rappaport, Konforti & Navon (1956) described a new medium for the isolation of salmonellas from infected material. This medium they claimed to be more effective in isolating salmonellas from faeces than selenite F broth or tetrathionate broth, provided that the inoculum was small. Three or four drops of a 1/1000 saline suspension of faeces in 5 ml. of the medium with subculture after 18 hr. at 37° C. on deoxycholate citrate agar was recommended. A larger inoculum was shown to yield fewer salmonellas.

In order to assess the efficacy of the new medium for routine use, a trial was carried out on faecal specimens in a laboratory which receives for examination material from a general hospital, general practitioners and the public health authorities. Deoxycholate citrate agar, selenite F broth and Rappaport's medium were inoculated in parallel when culturing all faecal specimens received for a period of one year.

METHODS

Deoxycholate citrate agar plates were prepared according to the method of Hynes (1942) and selenite F medium according to Leifson (1936). Rappaport medium was prepared as in the original formula (Rappaport *et al.* 1956) with the exception that magnesium chloride A.R. was used instead of magnesium chloride C.P. A noteworthy point is that in making Rappaport solution B the addition of 40 g. magnesium chloride to 100 ml. of water results in a total volume of 120 ml., giving a concentration of 33·3 %. The complete medium contains 10 ml. of solution B in 113 ml. This gives a final concentration of 2·9 % magnesium chloride and not 4·0 % as stated in the original paper.

Deoxycholate citrate agar was inoculated direct with a loopful of faeces. Approximately 0·5 g. of faeces was added to 10 ml. of selenite F broth, and 10 ml. of Rappaport medium was inoculated with 5 drops of a 1/1000 suspension of faeces in saline. After overnight incubation at 37° C., the fluid media were subcultured on deoxycholate citrate agar and incubated for a further 24 hr.

Non-lactose-fermenting colonies were picked and inoculated on urea agar slopes. Urease negative organisms were checked for agglutination with salmonella and shigella agglutinating sera supplied by the Standards Laboratory of the Public Health Laboratory Service. Biochemical properties were confirmed by using diagnostic plates prepared according to the formula of Knox (1949).

RESULTS

A total of 2476 specimens were examined and 99 isolations of salmonellas and shigellas were made, comprising 67 salmonellas and 32 shigellas.

Table 1 shows an analysis of salmonella isolations from the three media. Isolations from Rappaport medium were 91 % of the total compared with 37 % from direct plate culture and 78 % from selenite F. The number of salmonellas isolated from a combination of direct plating and selenite F enrichment was 53. The addition of Rappaport medium raised the total to 67. This represents an increase of 26 % in the number of salmonella isolations.

Table 1. *Salmonella isolations*

Total no. of isolations	Medium	No. of isolations	Percentage of total isolations
67 salmonellas	Deoxycholate citrate agar	25	37
	Selenite F broth	52	78
	Rappaport medium	61	91

No. of salmonella isolations excluding those from Rappaport culture = 53.

Table 2 shows in detail the distribution of positive cultures among the three methods of culture employed for each of the salmonella serotypes encountered. The last column represents the serotype distribution of the 13 isolations which would have been missed if Rappaport's medium had not been included. Omission of selenite F would have resulted in four fewer isolations.

Table 3 shows the isolation rate for shigellas in the same series. Of the 32 isolations only 12 (27 %) were recovered from Rappaport compared with 25 (78 %) both from selenite F and by direct plate culture. None of the positive Rappaport cultures was negative by the combination of the other two methods.

DISCUSSION

Rappaport *et al.* introduced their new enrichment medium to improve salmonella isolations from faeces, as they claim it allows unrestricted growth of salmonellas but inhibits the growth of coliform organisms. Collard & Unwin (1958), in a cultural survey of 1000 faecal specimens at Ibadan, raised the number of salmonella isolations from 16 to 26 by adding a single tube of Rappaport medium to their culture regime. This represents a 60 % improvement in salmonella isolations compared with 26 % in the series reported here, but since the two series are of unequal size comparison is difficult. Sen (1964) also employed Rappaport medium for faecal specimens and increased salmonella isolations by 14 %. It is clear, therefore, that the medium is of value.

Iveson, Kovacs & Laurie (1964) showed the new medium to be of great value in the isolation of salmonellas from contaminated coconut and demonstrated its superiority over both selenite F and tetrathionate broth.

No claim for efficacy in shigella isolation has been made with Rappaport medium. From the present series it appears that *Shigella sonnei* is inhibited by

Table 2. *Salmonellae and the media from which isolations were made*

Serotype	Total	Rappaport + selenite + direct D.C.A. +	Rapp. + sel. + D.C.A. −	Rapp. − sel. + D.C.A. −	Rapp. − sel. − D.C.A. +	Rapp. + sel. − D.C.A. +	Rapp. − sel. + D.C.A. +	Rapp. + sel. − D.C.A. −
Salmonella typhimurium	39	12	13	3	1	1	1	8
S. paratyphi B	3	—	2	—	—	—	—	1
S. heidelberg	1	—	1	—	—	—	—	—
S. reading	4	3	1	—	—	—	—	—
S. thompson	6	1	2	1	—	—	—	2
S. bareilly	1	—	1	—	—	—	—	—
S. bovis morbificans	8	4	3	—	—	—	—	1
S. chester	2	2	—	—	—	—	—	—
S. braenderup	3	—	2	—	—	—	—	1
All serotypes	67	22	25	4	1	1	1	13

Table 3. *Distribution of* Shigella *isolations*

Type	Total	Rappaport + selenite + direct D.C.A. +	Rapp. − sel. + D.C.A. −	Rapp. − sel. + D.C.A. +	Rapp. − sel. − D.C.A. +	Rapp. + sel. − D.C.A. +
Sh. sonnei	30	9	7	8	4	2
Sh. flexneri	2	1	0	0	1	0

the medium. Seven of the 32 shigellas were isolated from selenite F and not from direct D.C.A. plating. This is near the upper limit of the generally accepted improvement to be expected but may be due to the inclusion of some post-treatment specimens in the series. A further seven were isolated from direct plating but not from selenite F. Our shigella isolations were predominantly *Sh. sonnei*. No information concerning the efficacy of the various cultural methods for *Sh. dysenteriae*, *Sh. flexneri*, or *Sh. boydi* was obtained.

It is interesting to note that in the series described here the number of salmonellas isolated from direct plating on deoxycholate citrate agar is very low when compared with Rappaport medium or selenite F, especially as it was introduced as a selective medium for these organisms. Not only does deoxycholate citrate inhibit the coliform group but also to a lesser extent salmonellae. It could be argued that D.C.A. is too inhibitory for general use and it is an impression in this laboratory that efficient plating on MacConkey's agar medium yields as many pathogens, if not more, than on D.C.A. Two and a half times as many salmonellas were isolated after Rappaport enrichment and twice as many after selenite F than on direct plating. Our results seem to indicate that if salmonellas alone are being sought in faeces, primary plating on a solid medium is unnecessary if the two methods of fluid enrichment culture are employed. In the present survey only one of the 67 salmonellas would have been missed if direct plating had been omitted.

A factor which may have influenced the results of this series is that the size of the inoculum varied with each method, but the inocula were similar to the original recommendation in each case. Thomson (1954) and Armstrong (1954) showed an advantage in using small inocula in the plating of faecal specimens. The importance of using a dilute suspension for the magnesium chloride/malachite green medium has been emphasized by Rappaport *et al.* and the employment of this method might have had a bearing on our results.

SUMMARY

Rappaport's magnesium chloride/malachite green medium was employed in the cultural examination of 2476 faecal specimens in parallel with selenite F broth enrichment and direct plate culture on deoxycholate citrate agar. The use of the medium increased the salmonella isolation rate by 26% over the number of isolations from the other two methods, but proved of no additional value in the isolation of shigella organisms. The addition of Rappaport medium is recommended in routine faecal examination.

We wish to thank Dr R. J. Henderson, the director of the laboratory, for encouragement and helpful criticism in the preparation of this paper, and also other members of the laboratory staff who co-operated in the survey.

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J. Hyg., Camb. (1965), **63**, 497
Printed in Great Britain

Studies on the pathogenesis of rinderpest in experimental cattle

IV. Proliferation of the virus following contact infection

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(*Received* 8 *May* 1965)

Rinderpest can be transmitted easily to experimental cattle by many parenteral routes, but the natural method of infection remains unknown. Many text-books still state that infection takes place by the oral route in the ordinary course of events (Hutrya, Marek & Manninger, 1946; Henning, 1949; Davies, 1955; Blood & Henderson, 1960; Hagan & Bruner, 1961). This is in spite of evidence that drenching with large quantities of highly infectious material may fail to set up the disease (Schein & Jacotot, 1925; Hornby, 1926; Maurer, 1965) and, on the contrary, a great deal of information indicating the ease with which the virus invades the body when introduced via the upper respiratory tract (see Hornby, 1926; Hall, 1933; Maurer, 1965; Liess & Plowright, 1964; Plowright, 1964) or as an aerosol (Provost, 1958).

So far as we are aware, no systematic studies have hitherto been undertaken to demonstrate the route or routes by which virus invades the body in natural cases of rinderpest. Cattle were, therefore, killed at varying time intervals after controlled exposure to contact infection with a single virulent strain of virus and the infectivity in various tissues was determined by a tissue-culture technique. Particular attention was paid to parts of the upper and lower respiratory tracts, which previous investigations had indicated might provide a primary portal of entry. The results of this experiment were, in many respects, comparable to those obtained when cattle were infected by the intranasal instillation of virus (Plowright, 1964).

MATERIALS AND METHODS

Virus

The virulent RGK/1 strain of rinderpest (Liess & Plowright, 1964) was used throughout the experiment. At the time of its use the virus had undergone, in succession, two passages in primary calf kidney cells, one passage in cattle, an additional passage in primary calf kidney cells and, finally, three further passages in cattle. The animal utilized for the final passage (ox no. 9519) was killed on the morning of the 4th day of pyrexia, and portions of its spleen were stored at $-70°$ C.

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Experimental animals and their infection

All experimental cattle were grade steers or heifers, aged between 1 and 2 years and either Ayrshire or Friesian crosses. Their sera contained no rinderpest-neutralizing antibodies (Plowright & Ferris, 1961).

The animals were housed in an isolation unit, where rigid precautions were taken to prevent transfer of infection from stall to stall. Hay was fed from the floor and water was available from a single trough in each stall; hence, opportunities for the dissemination of virus in any one stall were very good.

To produce cases of contact infection a series of animals was injected parenterally and used as virus donors. Experimental infections were produced by the subcutaneous inoculation of 2 ml. of a 10^{-1} or 10^{-2} suspension of the spleen of ox no. 9519 in culture maintenance medium; such inocula contained approximately $10^{4\cdot8}$ or $10^{5\cdot8}$ TCD 50 of virus and always gave rise to pyrexia on the 3rd to 5th morning following inoculation.

It was shown previously that rinderpest-infected cattle excreted virus in their nasal secretions, urine and faeces, but quantitative data suggested that the nasal excretions constituted a major source of contagion for other animals. Further, the percentage of infected animals excreting virus by the nasal route reached a maximum on the 4th day of pyrexia and high virus titres were encountered in the nasal secretions between the 3rd and 7th days of fever (Liess & Plowright, 1964). Accordingly, groups of two or three susceptible animals were introduced to stalls containing a virus donor, which had attained the 3rd, 4th or 5th morning of its reaction. After 24 hr. exposure the susceptible animals were transferred to separate clean stalls. To avoid the possibility of any later infection, the donor animals were immediately removed from the unit and the stall used for contact exposure was disinfected.

Collection of materials

Cattle were killed and exsanguinated at daily intervals from the 2nd to the 10th mornings after their first contact with a virus donor. *Blood* was collected in every case for viraemia estimation (Plowright, 1964) and for separation of serum.

The technique for collection of solid tissues was as already described (Plowright, 1964). The *pharyngeal mucosa* was obtained, in all except four instances, by removing a 2–3 cm. section along the median fold of mucous membrane, at a point lying immediately ventral to the insertion of the ventral straight muscles of the neck; the median fold here comprises several smaller, longitudinal ridges lying in close apposition to each other and extremely rich in lymphoid tissue.

Unless otherwise stated the following tissues were always collected: *Nasal mucosa* was obtained from the middle third of the dorsal turbinate bone. The *upper tracheal mucosa* was dissected from the ventral aspect of the organ, over the first three cartilaginous rings, and the *lower tracheal mucosa* from between the opening of the right apical bronchus and the bifurcation. The *left diaphragmatic bronchial mucosa* was obtained by opening the main branch of the left bronchus along its entire length; the mucosa could then be removed in strips of up to 4 cm.

in length, by plucking at it with rat-toothed forceps. The mucosa of the *tongue* was removed from the under surface of the free anterior part of the organ, over an area where the epithelium is tightly bound.

One each of the following *lymph nodes* was excised intact: *submaxillary, pharyngeal, left prescapular, left costocervical, left bronchial* and *left prefemoral.* Except in one instance a single large, or several small, *middle cervical lymph nodes* were collected, from either side of the trachea, in the middle of the neck.

A portion of *spleen* and an entire *palatal tonsil* were removed, while *lung* tissue was also obtained from the diaphragmatic surface of the *left diaphragmatic lobe* and from the hilar region of the *left cardiac lobe.*

Subsequent treatment of tissues

Where necessary, specimens were dissected to remove capsular and trabecular connective tissue. The technique used in the preparation of tenfold dilutions of solid tissue has already been described; so, also, have the preparation of blood leucocyte fractions and blood dilution series (Plowright, 1964).

Each preparation was inoculated in 2 ml. amounts into each of five tubes of primary calf kidney (BK) cells. It was found previously (Plowright, 1964; Taylor & Plowright, 1965) that, for solid tissues, dilutions lower than 10^{-2} could produce cytotoxic changes in BK cells and hence this was the highest concentration inoculated.

It soon became apparent that many animals would be tested in which no virus would be detected. Further, it was not possible to anticipate from the day of slaughter the amount of virus that would be present in positive animals. Consequently, after some initial trials, dilutions were chosen which ranged from 10^{-2} to 10^{-4} or 10^{-5} for solid tissues, and from 10^{-1} to 10^{-2} or 10^{-3} for whole blood, with the invariable inclusion of a leucocyte fraction. This procedure inevitably resulted in a failure to reach titration end-points in cases where virus generalization was well established, but for the present purpose such animals were of reduced significance.

Preparation and maintenance of cell cultures

The production, maintenance and post-inoculation treatment of BK cultures was as already detailed. Final microscopic observations were made on the 9th day, except in the case of the tissues of ox no. 9927, when bacterial contamination made it necessary to terminate readings on the 7th day. Titres were calculated as before (Plowright, 1964).

Serum neutralization tests

Sera collected at the time of slaughter were screened for rinderpest-neutralizing antibodies by the method of Plowright & Ferris (1961); the test dose of virus was $10^{1.6}$ TCD50 per tube.

RESULTS

Clinical and post-mortem observations

Two animals (nos. 9771 and 9776) were killed on the first morning of pyrexia; in both of these virus was recovered in large amounts from many tissues (see

Tables 1 and 2). No fever or other clinical abnormalities were observed in the remaining cattle exposed to contact infection. Lymph node congestion was noted in two animals (nos. 9997 and 9931), in both of which virus had become generalized.

Virus proliferation

A total of 35 animals were investigated and virus was detected in only 15 of them. The distribution of virus in the tissues of these 15 animals is given quantitatively in Tables 1 and 2.

Table 1. *The titre of virus in the tissues of cattle killed on days 3–6 following contact exposure to rinderpest*

Time after exposure ...	3 days		4 days		5 days	6 days			
Animal no. ...	9927*	9999	9975	9997	9925	9762	9766	9771	9931
Nasal mucosa	0·0	0·0	0·0	0·0	0·0	0·0	2·4	0·0	0·0
Pharyngeal mucosa	0·0	0·0	0·0	⩾ 4·2	3·8	0·0	⩾ 6·2	6·7	4·6
Upper tracheal mucosa	0·0	0·0	0·0	1·8	4·4	0·0	⩾ 4·2	6·4	2·4
Lower tracheal mucosa	0·0	0·0	0·0	2·4	2·6	0·0	⩾ 4·2	6·0	2·7
Bronchial mucosa	0·0	0·0	0·0	1·8	2·2	0·0	⩾ 4·2	4·6	3·0
Lung—hilar	0·0	0·0	0·0	Tr†	0·0	0·0	⩾ 4·0	3·0	1·6
Lung—diaphragmatic	0·0	0·0	0·0	Tr	0·0	0·0	⩾ 4·2	3·8	Tr
Pharyngeal lymph node	2·2‡	3·4	1·6	⩾ 4·2	3·8	2·4	⩾ 6·2	6·6	4·4
Mid-cervical lymph node	0·0	0·0	0·0	⩾ 4·2	3·4	1·7	⩾ 5·2	6·0	2·4
Costocervical lymph node	0·0	0·0	0·0	3·4	⩾ 5·0	3·2	⩾ 6·2	6·2	2·8
Bronchial lymph node	0·0	0·0	3·0	3·8	4·4	0·0	⩾ 6·2	6·7	3·6
Tongue mucosa	0·0	0·0	0·0	0·0	0·0	0·0	1·6	1·6	0·0
Submaxillary lymph node	0·0	0·0	0·0	3·8	4·0	2·6	⩾ 6·2	6·4	5·0
Tonsil	2·8	0·0	0·0	⩾ 4·2	⩾ 5·2	2·6	⩾ 6·2	7·6	3·4
Blood	0·0	0·0	0·0	0·7	1·2	0·4	⩾ 2·2	2·6	1·4
Spleen	0·0	0·0	Tr	⩾ 4·2	4·2	2·4	⩾ 6·2	6·2	2·8
Prescapular lymph node	0·0	0·0	0·0	⩾ 4·0	3·6	1·6	⩾ 6·2	6·2	3·4
Prefemoral lymph node	0·0	0·0	0·0	3·4	3·4	0·0	⩾ 6·2	6·2	0·0

* Observations for ox no. 9927 terminated on day 7 owing to bacterial contamination of cultures.

† Tr = Trace, i.e. one tube was infected of five inoculated with 10^{-2} dilution of solid tissue.

‡ Titre expressed as $\log_{10}$ TCD 50/g., or per ml. (blood).

Whereas no virus was detected in one animal killed on the second day following exposure, two of five destroyed on day 3 did show virus multiplication. Of these, no. 9927 had moderate amounts of virus in both the tonsil and pharyngeal lymph node, whereas in no. 9999 virus was found only in the latter (Table 1).

On day 4 virus was recovered from two of the six cattle tested. One of them (no. 9975) showed virus in the pharyngeal and bronchial lymph nodes, while a low-grade viraemia must also have occurred, as traces of virus were recovered from the spleen. In the other animal that was positive at this time, no. 9997, the

virus had become generalized, as evidenced by its presence in the blood, prescapular and prefemoral lymph nodes, etc.

Of the six animals killed on day 5, infectivity was demonstrated in the tissues of one only (no. 9925) and in this individual virus had also generalized. On day 6, virus was recovered from four of the six cattle tested. In three, nos. 9766, 9771 and 9931, the virus was widely disseminated; no. 9762 showed viral multiplication in the pharyngeal, mid-cervical, costocervical and submaxillary lymph nodes; also in the tonsil, blood, spleen and prescapular lymph node.

Table 2. *The titre of virus in the tissues of cattle killed on days 7–10 following contact exposure to rinderpest*

Time after exposure ...	7 days			8 days		10 days
Animal no. ...	9760	9779	9870	9776	9781	9757
Nasal mucosa	0·0	2·0	0·0	1·8	0·0	0·0
Pharyngeal mucosa	N.T.	⩾ 4·2	⩾ 4·2	N.T.	N.T.	N.T.
Upper tracheal mucosa	0·0	⩾ 2·2	1·6	⩾ 3·2	0·0	0·0
Lower tracheal mucosa	0·0	⩾ 2·2	2·2	⩾ 3·2	0·0	0·0
Bronchial mucosa	0·0	⩾ 2·2	⩾ 3·2	⩾ 3·2	0·0	0·0
Lung—hilar	0·0	⩾ 3·2	0·0	⩾ 4·2	0·0	0·0
Lung—diaphragmatic	0·0	⩾ 3·2	2·0	⩾ 4·2	0·0	0·0
Pharyngeal lymph node	2·4*	⩾ 5·2	⩾ 5·2	⩾ 5·2	2·2	2·4
Mid-cervical lymph node	Tr†	⩾ 4·2	4·0	⩾ 5·2	1·8	0·0
Costocervical lymph node	0·0	⩾ 4·2	2·8	⩾ 5·2	N.T.	0·0
Bronchial lymph node	0·0	⩾ 5·2	4·8	⩾ 6·2	3·8	0·0
Tongue mucosa	0·0	1·8	0·0	0·0	0·0	0·0
Submaxillary lymph node	0·0	⩾ 5·2	4·4	⩾ 5·2	2·0	2·0
Tonsil	3·2	⩾ 5·2	4·4	⩾ 5·2	1·8	0·0
Blood	0·0	⩾ 3·0	Tr	2·6	Tr	Tr
Spleen	1·6	⩾ 5·2	3·8	⩾ 5·2	Tr	1·6
Prescapular lymph node	0·0	⩾ 4·2	2·4	⩾ 5·2	0·0	0·0
Prefemoral lymph node	0·0	⩾ 4·2	3·2	⩾ 4·2	0·0	0·0

* Titre expressed as $\log_{10}$ TCD 50/g., or per ml. (blood).

† Tr = Trace, i.e. one tube was infected of five inoculated with a 10^{-2} dilution of solid tissue, or by a blood leucocyte fraction.

N.T. = Not tested.

Four animals were killed on day 7, of which three yielded virus; in two, nos. 9779 and 9870, generalization had occurred, while in the third (no. 9760) virus was present in the tonsil, pharyngeal and mid-cervical lymph nodes. The recovery of virus from the spleen of this animal indicated a low-grade viraemia.

On day 8 the tissues of four cattle were harvested and in two instances virus was demonstrable. Generalization had occurred in no. 9776, but in no. 9781 virus was restricted to the pharyngeal, submaxillary, mid-cervical and bronchial lymph nodes, the tonsil, blood and spleen (Table 2).

No virus was recovered from either of the two animals killed on day 9, whereas no. 9757, sacrificed on day 10, had virus in its pharyngeal and submaxillary lymph nodes, blood and spleen.

Serum neutralization

As expected, no rinderpest-neutralizing antibody was detectable in the post-exposure sera of any of the experimental animals.

DISCUSSION

It was more difficult than expected to produce regular cases of rinderpest in cattle by 24 hr. contact exposure to single donor animals in the 3rd–5th days of the disease reaction. The infection in the recipients had sometimes become generalized in as little as 4 days, whereas in other instances no virus could be detected in selected tissues after 9 days. Liess & Plowright (1964), using the same strain of virus, at approximately the same passage level, noted regular contact infection after periods of 8–11 days with a mean of 8·6 days for 11 animals; in these cases the intensity of exposure during the first 2 days of pyrexia in the donor(s) was probably low or even negligible, increasing with the development of the disease in the donor(s).

The quantity of virus escaping in the nasal or other excretions of infected cattle may vary considerably from animal to animal. Thus, Liess & Plowright (1964) failed to demonstrate virus at any time in the nasal secretions of two out of 24 cattle, whilst urinary and faecal excretion was even more irregular. Such observations may well account for the fact that five donor cattle in these experiments failed to produce demonstrable infection in either of the two animals which were housed with them and killed 2–6 days later; they would also explain the great variation in the rapidity of development of the infection in the present experiments, assuming that the length of the eclipse phase depends on the dose of virus received.

A comparable irregularity of contact transmission with some Indian strains of rinderpest virus was reported by Cooper (1932), who found that exposure periods of 10 days sometimes failed to convey the infection to susceptible cattle, while other animals in continuous contact with presumed virus excretors did not react for 31–33 days.

Among the 15 animals from which virus was recovered, 13, unfortunately, had a viraemia at the time of sampling or must already have circulated virus, since it was demonstrable in the spleen. However, in five of these cattle dissemination was limited, as shown by the failure to obtain virus from the mucosae of the respiratory tract, lung parenchyma and prefemoral lymph node. Titration data for these five and two positive animals killed on day 3 are assembled in Table 3 and will serve as a basis for the following discussion.

The pharyngeal lymph node was invariably involved and this receives lymph from the tongue, floor of the mouth, hard and soft palates and gums, all covered by stratified squamous epithelium and thus unlikely to represent the primary portal of virus entry—at least when uninjured. Also draining to the pharyngeal lymph node are the mucosae of the pharynx and posterior part of the nasal cavity, the maxillary and palatine sinuses and the larynx (Sisson & Grossman, 1953).

The submaxillary lymph node was infected in three instances, the titre of virus being low and comparable in each case to that in the pharyngeal node of the same animals; this node receives afferent vessels from the muzzle, lips, cheeks, gums, hard palate and tip of the tongue—all provided with stratified squamous epithelium—the anterior parts of the turbinate bones and septum nasi. The evidence from the two cephalic nodes, even in the absence of virus proliferation in the mucosae, gives support to the hypothesis that part, at least, of the infecting virus entered through the nasal mucosae or, less probably, the associated sinuses and pharynx.

Table 3. *Results for animals which gave indications of the route(s) of infection*

Time after exposure ...	3 days		4 days	6 days	7 days	8 days	10 days
Animal no. ...	9927*	9999	9975	9762	9760	9781	9757
Nasal mucosa	—	—	—	—	—	—	—
Pharyngeal mucosa	—	—	—	—	N.T.	N.T.	N.T.
Upper tracheal mucosa	—	—	—	—	—	—	—
Lower tracheal mucosa	—	—	—	—	—	—	—
Bronchial mucosa	—	—	—	—	—	—	—
Lung—hilar	—	—	—	—	—	—	—
Lung—diaphragmatic	—	—	—	—	—	—	—
Pharyngeal lymph node	2·2	3·4	1·6	2·4	2·4	2·2	2·4
Mid-cervical lymph node	—	—	—	1·7	Tr†	1·8	—
Costocervical lymph node	—	—	—	3·2	—	N.T.	—
Bronchial lymph node	—	—	3·0	—	—	3·8	—
Tongue mucosa	—	—	—	—	—	—	—
Submaxillary lymph node	—	—	—	2·6	—	2·0	2·0
Tonsil	2·8	—	—	2·6	3·2	1·8	—
Blood	—	—	—	0·4	—	Tr	Tr
Spleen	—	—	Tr	2·4	1·6	Tr	1·6
Prescapular lymph node	—	—	—	1·6	—	—	—
Prefemoral lymph node	—	—	—	—	—	—	—
Probable route of infection	UR	UR	LR	UR(?) LR	UR(?)	UR	LR UR UR

Titre expressed as $\log_{10}$ TCD 50/g., or per ml. (blood).
* Observations for ox no. 9927 terminated on day 7, owing to bacterial contamination.
N.T. = Not tested.
† Tr = Trace.
— = virus not detected in 0·1 g. tissue or leucocytes from 13 ml. blood.
UR = upper respiratory tract. LR = lower respiratory tract.

Four animals showed very early proliferation of virus in the palatal tonsil, which drains only its own epithelium, all of stratified squamous type and lining the crypts. It is interesting to note that two cattle which showed virus in the tonsil (nos. 9927 and 9762) had no detectable infection of the pharyngeal mucosa, which has a comparable histological structure of stratified squamous epithelium, closely associated with nodular lymphoid tissue. In discussing the vulnerability of the tonsil to microbial infections, Payling Wright (1954) observed that the epithelium lining the depths of the tonsillar crypts presents certain structural weaknesses, in the form of thinning or even actual defects (Stöhr's lacunae).

He also admitted that it was not known whether leucocytes could wander out into the crypts, engulf pathogenic organisms and return to the tissue of the tonsil. Either explanation could account for this structure being a primary site of proliferation of rinderpest virus.

In two cases (nos. 9975 and 9781) the highest titre was recorded in the bronchial lymph node, which contained more than 20 times as much virus as any other structure. The spleen showed evidence of early generalization in each of these animals but it seems reasonable to suppose that the lung was the site of entry for virus which passed to the local lymph node, without undergoing detectable multiplication in either the bronchial mucosa or the lung parenchyma. In ox no. 9762, the greatest quantity of virus was found in the left costocervical lymph node. Again, there was evidence of early generalization and an absence of virus from the associated tracheal and bronchial mucosae; nevertheless, the indications were that infection had occurred through these surfaces. The occurrence of virus in the mid-cervical nodes of three of the animals in Table 3 may not have implied primary multiplication there, since the titre was very low in each case and in the same animals extension to the spleen had already occurred, presumably following a low-grade viraemia.

The results presented in Table 3, admittedly small in numbers, would support the hypothesis that rinderpest infection is naturally acquired via the mucosae of the upper and also, in some instances, via the lower respiratory tract. It is of interest to observe that the experimental susceptibility of the lower respiratory tract was reported by Hornby (1926), who infected cattle by the intratracheal inoculation of infectious material. Primary proliferation cannot be demonstrated at the presumed sites of mucosal penetration but virus is rapidly transported to the local lymph node and either multiplies there or passes very quickly through into the circulation, giving rise to a low-grade viraemia and localization in the spleen or other lymphopoietic structures. This theory is also supported by the data of Plowright (1964), who could not demonstrate primary proliferation of virus in the nasal mucosae of animals infected by intranasal instillation.

Some of the moderately high titres found in these experiments for virus in the pharyngeal, tracheal and bronchial mucosae of cattle which were still in the incubation period of the disease (see, for example, nos. 9766, 9997 and 9925) offer a possible explanation for the finding that the nasal excretions may occasionally become infective as early as the 2nd day preceding pyrexia (Liess & Plowright, 1964). It does not follow, of course, that high virus content in a mucosal tissue will necessarily imply excretion from the relevant surface; this has already been shown to be a false assumption in poxvirus infection of the respiratory tract of the rabbit (Bedson & Duckworth, 1963). It is also noteworthy that, whereas, in cattle infected by intranasal instillation, virus was not detected in the nasal mucosae until the 2nd day of fever, three of 13 animals in these experiments (nos. 9766, 9779, 9776) did have small quantities of virus in this situation before the onset of pyrexia.

So far as we are aware, the only comparable experimental study of naturally acquired cases of a viral exanthem is that of Bedson & Duckworth (1963), who

investigated rabbit pox. They encountered similar difficulties in infecting all their animals, since 12 of 31 did not yield virus at the time of sampling. However, in their experiments there was a tendency for rabbits killed later after exposure to show a wider dissemination and higher titres of virus than those killed earlier; this, surprisingly, was not our experience with naturally acquired rinderpest. Bedson & Duckworth (1963) encountered cases of both upper and lower respiratory tract infection, but in many instances a complete 'primary complex' was established, with virus proliferation both at the presumed surface of entry and in the regional lymph node.

SUMMARY

Cattle were infected with rinderpest virus by housing them for 24 hr. in stalls containing donor animals which had been reacting to the disease for 3–5 days. They were then transferred to individual clean stalls and killed on the 2nd to 10th days following first exposure. Various tissues were collected, particularly those of the upper and lower respiratory tracts, and their virus content was estimated in calf-kidney tissue cultures.

Virus was recovered from 15 of 35 animals tested and in eight of these generalization had occurred, although only two had begun to show a pyrexial response. The stage of the infection could not be predicted from the time that had elapsed following exposure, since early, limited proliferation was encountered on the 3rd to the 10th days.

It was considered that seven animals gave indications of the pathways by which natural infection had occurred. In each of these virus proliferation was established very early in the pharyngeal lymph node; in three the submaxillary lymph node was similarly involved and in four the palatal tonsil. It was suggested that these data probably indicated that infection always occurred via the upper respiratory tract.

In three cases virus titres were highest in the bronchial or costocervical lymph nodes; this was construed as evidence for the additional involvement of the lower respiratory tract in primary infection.

No infectivity could be demonstrated in the mucosae or lung parenchyma associated with the above-mentioned lymph nodes and this, together with previously published data, was accepted as strong presumptive evidence that the infecting virus passes through the mucosae without producing a local lesion or proliferating there. These results were compared briefly with those of Bedson & Duckworth (1963) for rabbit pox.

The authors wish to express their gratitude to the junior staff who gave much valuable assistance. In particular we would like to thank Mr Francis Ngugi, Mr Harrison Agili and Mr Joash Adero.

This paper is published by permission of Mr H. R. Binns, C.M.G., O.B.E., Director of the East African Veterinary Research Organization.

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J. Hyg., Camb. (1965), **63**, 507
Printed in Great Britain

Ringworm carriage and its control in mice

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(*Received* 17 *June* 1965)

INTRODUCTION

The carriage of *Trichophyton mentagrophytes* (Robin) Blanchard by symptom-free laboratory mice has been described by La Touche (1957), Dolan, Kligman, Kobylinski & Motsavage (1958) and Mackenzie (1961). It may often be only the development of infections in the animal handlers which draws attention to the carriage of the fungus by the mice. These ringworm infections in man may take the form of a rapidly developing eczematous and vesicular lesion and the mouse carrier constitutes a significant hazard to the laboratory animal worker.

In radiobiological and oncogenic experiments where the animal may be studied throughout its life span, killing infected mice as suggested by Parish & Craddock (1931) is impracticable and the adoption of control measures is essential.

The study to be described was initiated when six out of thirteen workers in contact with a colony of about 1500 mice belonging to a radiobiological laboratory developed ringworm (Fig. 1). In each case *T. mentagrophytes* of the mouse variety was isolated from skin scrapings taken from the patient's lesions. Less than 1 % of the mice showed any signs of fungal infection.

MATERIALS AND METHODS

Animals

The mouse colony studied was of two strains, an inbred C_3H/Bi colony and a smaller breeding nucleus of BALB/c mice, randomly bred within the closed colony. The mice were kept at 65° F. in a small, air-conditioned room, and no mice were introduced from outside.

Ten other strains (A, AH, AKR, CBA, C 57 Bl, C 57 Br, C 57 L, DBA, ICI and Swiss mice) were examined in other laboratories.

Caging of mice

Breeding mice were housed in galvanized metal carriers at one end of the room, together with weaned litters. After 3 weeks, the litters were ear-marked and transferred to mesh drawers hanging in a battery rack, where they remained until placed in metal carriers again for experimental use. Breeding and experimental mice were kept in the same room since space did not allow any other arrangement and in general the breeding mice did well. The total number of mice in the room averaged 1500, with 50–60 breeding C_3H pairs, and variable numbers in stock and

experiments during the year. The numbers of the BALB/c mice in stock were always smaller, and work in clearing up the outbreak of ringworm was concentrated on the more valuable inbred stock.

Culture technique

The number of mice carrying the fungus was determined by rubbing a Petri dish of malt-extract agar (containing 40 μg./ml. chloramphenicol and 0·5 mg./ml. cycloheximide) over the backs of the mice. The plates were incubated at 25° C. and the numbers of colonies of *T. mentagrophytes* which developed were counted from 5 to 7 days onwards.

Incidence of ringworm carriage in the colony

Random samples of approximately 10% of the mice were examined at 6-monthly intervals from January 1962 to December 1964. In addition, groups of mice representing the different categories of animal within the colony were investigated to determine the effect of age, length of time spent in the stock rack and irradiation upon the level of carriage. Evaluation of the weight of infection in experimentally treated groups was made by examining each mouse individually at weekly intervals, and counting the number of colonies of *T. mentagrophytes* isolated per mouse sweep plate. This gave both the percentage of mice in the group carrying the fungus, and the weight of carriage among the carriers.

Dipping technique

The dip used was the acaricidal dip described by Bateman (1961): this is a mixture of 2 g. DMC (di-*p*-chlorophenyl methylcarbinol, Sherwin Williams Inc., Cleveland, Ohio) dissolved in 3 g. ethanol, and 67 g. Tetmosol (25% tetraethylthiuram monosulphide in industrial alcohol, I.C.I. Ltd., Wilmslow, Cheshire) made up to 1 l. with warm tap water. The dip is kept at 37° C., and the mice are pulled through the dipping bath for 5–10 sec., so that the whole animal is immersed except for the tip of the muzzle. The animals are then momentarily completely immersed. After 10 min. the procedure is repeated; the first dip wets the fur and the second penetrates it. The double treatment is repeated after an interval of 3 weeks. This procedure is quite harmless to young mice provided that they are kept warm whilst drying, but is not without risk to mice over 1 year old, since some have died, apparently of cold, afterwards.

Antifungal activity of the dip constituents

Tetmosol and DMC were separately suspended in distilled water in the concentrations used in the complete dip, i.e. 1·7% tetraethylthiuram monosulphide and 0·2% DMC, and inoculated with 0·02 ml. of a suspension containing 10^5 spores of *T. mentagrophytes* per ml. After shaking for ½, 1 and 2 hr. drops were removed and streaked out on malt-extract agar.

Tetmosol and DMC in peptone glucose broth in the above concentrations and in tenfold dilutions were inoculated with 0·02 ml. of the same suspension of *T. mentagrophytes* and the minimum inhibitory concentrations determined.

Human hair samples were soaked in Tetmosol and DMC in the dip concentrations for 1 hr., the solutions were filtered off and the hair was air-dried. Samples of hair were placed in 25 ml. universal containers containing sterile distilled water and inoculated with a dense suspension of *T. mentagrophytes*. Untreated hair in distilled water was similarly inoculated as a control. The samples were incubated at 25° C. and examined at different time intervals after inoculation.

Level of mite infestation

The older mice in the closed colony were infected with mange mites, both *Myobia musculi* (Shrank) and *Myocoptes musculinus* (Koch). The degree of infestation was determined by the method of Tuffery and Broach (personal communication). With flamed forceps equal-sized tufts of hair were plucked from the head, back and belly, and examined in liquid paraffin under low magnification (× 100). The degree of infestation was judged on an arbitrary scoring method:

0	No trace	5–6	Dead mites
1–2	Empty eggs only seen	7–8	Live mites
3–4	Live eggs		

The scores were added at each site, to give a maximum of 20, with a total maximum per mouse of 60.

Experimental transfer of mites and ringworm

An attempt was made to transfer both mites and ringworm to non-infected animals by boxing them with mice known to carry both. Old, heavily infected experimental mice were placed with young C_3H weanlings free from both infestations. Mice were boxed in groups of five, either two donors to three recipients or one to four. Individual mite and colony counts were made on each mouse at weekly intervals.

RESULTS

In the initial survey of the colony, the fungus was isolated from over 90 % of a 10 % random sample of C_3H/Bi stock and a slightly lower percentage of the BALB/c. Whilst all except the very youngest mice were carriers, less than 1 % showed any signs of infection.

Clinical symptoms in the stock mice differed in the two strains. The agouti C_3H mice developed bald patches, usually on the back or belly, but culture of tail skin scrapings in this strain was negative. The BALB/c mice, unless experimentally irradiated, never showed hair loss, but some developed raised brown circular scabs on the tail, from which *T. mentagrophytes* was isolated. La Touche (1957) found that in two unnamed strains of mice fungal carriage on the tail was an important factor in maintaining infection in a colony.

Effect of dipping

As both strains of mice in this colony were also infested with mites, it was thought that the fungus might find a more favourable environment on mouse skin that had been abraded by mite bites. As a first step in cleaning up the colony

an acaricidal dip (Bateman, 1961) was used. This not only cleared the mice of mites but reduced fungal carriage (Davies & Shewell, 1964).

Since April 1963 breeding C_3H pairs have been routinely dipped before pairing, and from December 1963 experimental mice for long-term experiments have been dipped before being used. The success of these measures in reducing carriage of fungus and decreasing the risk to those handling the mice is shown in Fig. 1.

Only one worker became infected after the breeding stock were dipped, and there have been no infections since mice for long-term experiments have been dipped before being used. The overall incidence of mice carrying the fungus has

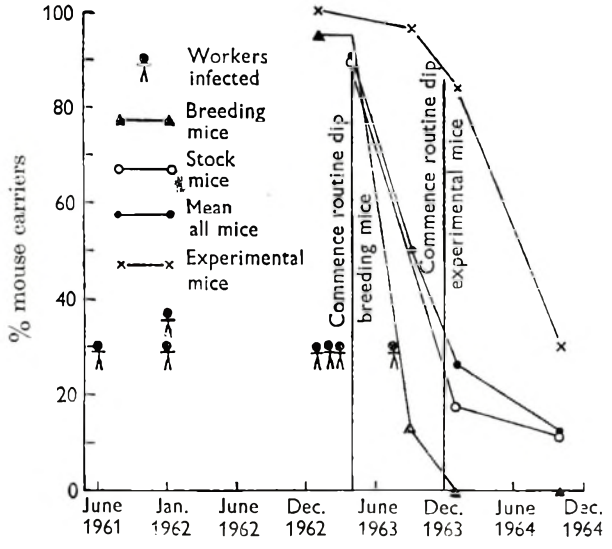

Fig. 1. Effect of routine dipping of all breeding mice from April 1963 and mice for experiment from December 1963 onwards.

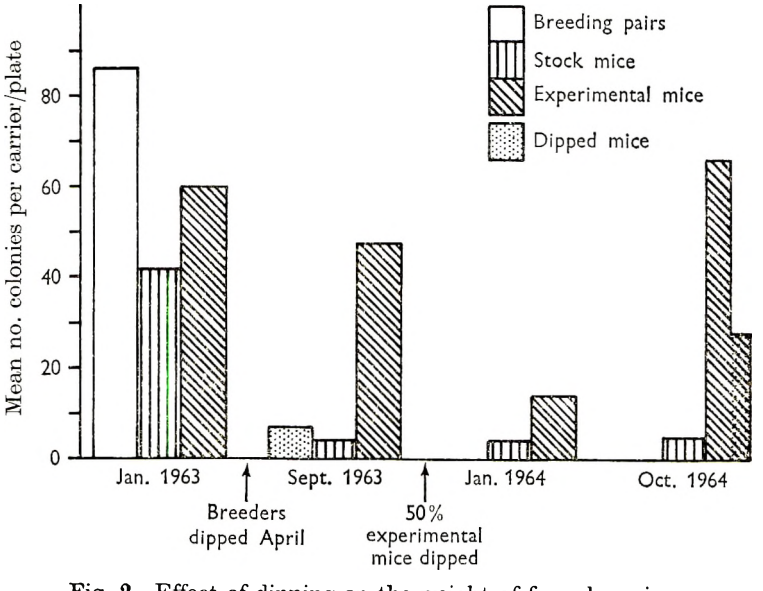

Fig. 2. Effect of dipping on the weight of fungal carriage.

ultimately been reduced to only 12 % despite the presence in the room of experimental mice from long-term irradiation experiments which are heavily infected, but were considered too valuable to risk dipping.

Figure 2 shows the effect of dipping on reducing the weight of fungal carriage in the different groups of mice in the colony. It may be seen that the degree of carriage varied among the different groups of mice. Thus in October 1964, although 11 % of the stock mice (made up of mice born to dipped parents but not themselves dipped) were carriers, the mean number of colonies isolated from individual mice carrying fungus was only 5/plate (Fig. 2) while the undipped experimental mice had a mean count of 66 colonies/plate.

Variations in carriage of undipped mice

(*a*) *With age*

Table 1 shows the incidence of carriage in stock mice of different ages before any control measures were introduced. The percentage of carriage among the stock mice increased with increasing age (or increasing time spent in the mesh drawers). There was no sex difference in carriage rate.

Table 1. *Incidence of fungal carriage in C_3H stock mice*

Age	No. examined	Percentage mice carrying fungus
3 weeks	8	25*
8 weeks	12	66
12 weeks	12	75
3–6 months	12	92
6 months–1 year	12	100
Over 1 year	10	100

* Figure not based on a random sample, but that found in the weanlings of two litters where both parents were carriers.

(*b*) *Effect of whole-body irradiation*

Some of the experimental mice are the long-term survivors of whole-body irradiation experiments: these animals have always been found to yield higher colony counts than non-irradiated carriers of comparable age. For example, the mean colony count $\pm$ S.E. of irradiated undipped 13-month-old mice in December 1963 was $34 \pm 7{\cdot}5$ while that of undipped 13-month-old stock mice was $16 \pm 3{\cdot}2$ ($P < 0{\cdot}001$).

Antifungal activity of the dip constituents

The action of the dip in reducing the frequency and weight of fungal carriage might have been due to the removal of the mites, to antifungal activity, or to both. The compounds DMC and Tetmosol were tested separately on infected mice: both were found to be antifungal and DMC slightly the more effective (Davies & Shewell, 1964). In a more critical evaluation of antifungal activity, it was found that no colonies of *T. mentagrophytes* grew on malt-extract agar after 7 days

incubation, when the inoculating suspension had been shaken with either DMC or Tetmosol in the concentrations in which they were used in the complete dip. The shortest period of shaking tested, 30 min., was enough to prevent growth. Suitable dilutions of samples from the higher concentrations of both compounds grew *T. mentagrophytes* when transferred to malt-extract agar, showing these compounds to be fungistatic in their activity. The minimal inhibiting concentrations were found to be 0·01 % for the tetraethylthiuram monosulphide and 0·1 % for DMC.

Fungal growth on human hair samples after 10 days incubation was only visible to the unaided eye on the untreated hair. Both the DMC and Tetmosol-soaked samples appeared to be clear. After 6 weeks' incubation, however, when examined microscopically, the characteristic pitting due to the development of *T. mentagrophytes in vitro* was evident in the treated as well as the untreated hair. The frequency of the pits was greatest in the untreated hair.

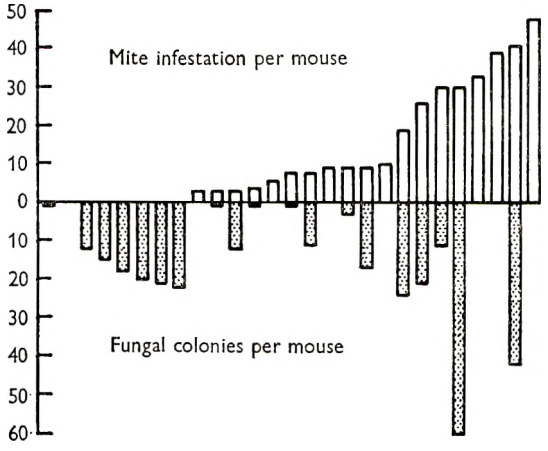

Fig. 3. Sample of twenty-seven stock mice showing level of mite infestation and number of colonies of *T. mentagrophytes* developing per mouse sweep plate.

Relationship with mite infestation

There was no correlation between the carriage of fungus and the presence of mites in the untreated stock ($\chi^2 = 0{\cdot}22$, $0{\cdot}7 > P > 0{\cdot}6$). Some mice carried the fungus and were free of mites on repeated examination; others had high mite scores and no fungus (Fig. 3). However, the highest concentrations of fungus were found on mice with mites, thus providing some support for the hypothesis that mite-abraded skin provides good lodgement for fungal multiplication.

Though it has been shown that irradiated mice gave significantly higher fungal colony counts than non-irradiated animals of the same age, there was no significant difference in mite score between the two groups (mean $\pm$ S.E. for irradiated mice = $13 \pm 2{\cdot}7$, non-irradiated mice = $13 \pm 2{\cdot}9$).

Experimental transfer of mites and fungus

No difference in transfer was found when there were two donors to three recipients, or one to four (Fig. 4 showing the pooled results).

A week after boxing the mice together, mites were found on, and a few fungal

colonies isolated from, the recipient mice. Although the mite count on the recipient mice increased until, by 4 weeks, it equalled that of the older mice, there was no comparable increase in fungal carriage which remained low throughout. When the experiments were repeated similar results were obtained showing the transmission of ectoparasites from one mouse to another does not parallel the carriage of ringworm spores.

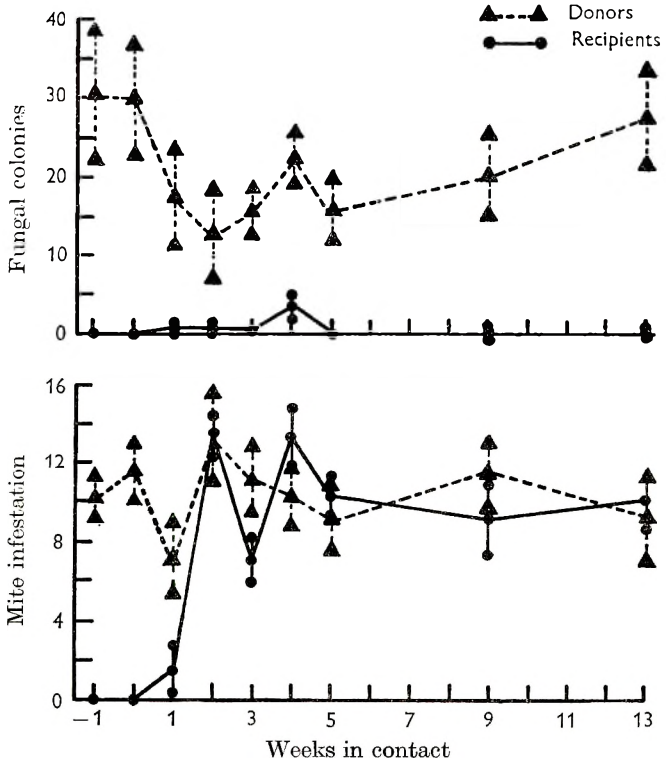

Fig. 4. Effect of contact on the transfer of mites and *T. mentagrophytes* from carriers to non-carriers during a 13-week period in boxes. (Points given represent mean values ± standard error.)

Incidence in other laboratories

During the past 2 years, as opportunity arose, we examined mice for fungal carriage in other laboratories and from commercial sources; the strain of mouse, incidence and weight of carriage and degree of human infection, when known, are given in Table 2. Mice were purchased on three occasions from source 1 which had a low incidence of light carriage, 2–3 % of the mice being carriers and an average of three or four colonies being isolated per mouse sweep plate. When the incidence of carriage is high, however, the weight of carriage may also be high, and colonies of these mice constitute a hazard for the handler. In the C_3H mouse colony described, when 88 % of the breeding stock were fungal carriers, and the mean colony count ± S.E. per mouse sweep plate was 61 ± 8, three workers developed ringworm within 3 months. In the Leeds colony with a 100 % carrier rate and a mean carriage weight of 103 ± 21 colonies per mouse sweep plate, both the regular animal handlers were infected within a 4-month period.

Table 2. *Incidence and weight of fungus carriage by mice in relation to proved human infections*

Source	Mouse strains	Pre-valence of mouse carriers (%)	Wt. of carriage, mean no. cols./ mouse	Human cases
L.A.C.	A, AKR, CBA, C57Br, A2G, C57Bl, C57L, DBA	0	0	None for at least past 6 years
W.F.I.	ICI	0	0	None
Source 1	Swiss	2	3	Not known
2	Swiss	8	2	Not known
3	C57Bl	90	4	Not known
S.M.H.M.S.	C_3H, BALB/c	90	61	Three cases in 4 months
Leeds (Fox: Leeds)	CBA, AH, C57Bl	100	103	Two cases in 4 months

DISCUSSION

The acaricidal efficacy of the combined DMC-Tetmosol dip has been clearly demonstrated by Bateman (1961) and Whiteley & Horton (1962). The present study shows that both the active acaricidal constituents are also fungistatic. When tested *in vitro* the antifungal activity of Tetmosol was greater than that of DMC by a factor of 10, but *in vivo* DMC appeared to be the more effective. This may be explained by the physical effect of DMC on the animals' fur. Mice dipped in DMC or a mixture of Tetmosol and DMC feel greasy to the touch for from 5 to 7 days after dipping and their fur stands up in peaks; the fur of mice dipped in Tetmosol alone, however, is groomed to normal appearance in 3–4 days.

DMC, a tertiary alcohol, is commercially available as a yellow wax, insoluble in water, soluble in alcohol but precipitating out of alcoholic solutions of less than 50%. Although the alcoholic concentration of the complete dip is approximately 20% the DMC appears to remain in suspension and this may be due to adsorption on the sulphide precipitated when the Tetmosol is mixed with warm water.

The antifungal effect of the dip would appear to be independent of its acaricidal action, since no relationship has been demonstrated between ectoparasite and fungal infection.

It has been shown that in mouse colonies where the incidence of carriage is high, the weight of carriage may also be high, and it is colonies of this type that are associated with infection in those handling the mice. Sweeping the back of a mouse with a Petri dish of malt-extract agar is a simple technique and we suggest that when the mean carriage per mouse rises to ten or more colonies there is an unnecessary hazard for the animal handler which may be eradicated with the dip described.

The mice in this investigation are maintained by a grant from the British Empire Cancer Campaign. We are grateful to those colleagues who allowed us to examine

their mouse stocks, in particular to Mr A. A. Tuffery, then of the Laboratory Animal Centre, Carshalton, and Mr Miles Fox, F.R.C.S., of the Renal Research Unit, Leeds.

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J. Hyg., Camb. (1965), **63**, 517
Printed in Great Britain

The bacteriology of tropical pyomyositis in Uganda

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(*Received* 26 *June* 1965)

Tropical pyomyositis is a condition which is clinically well defined (Ashken & Cotton, 1963; Cook, 1963). It consists essentially of large abscesses in voluntary muscles. They may be single or multiple and sometimes as much as a pint of pus may be evacuated from a single abscess. The condition has been reported from widely scattered parts of the tropical world including various parts of West Africa, East Africa, Malaya, Dutch East Indies, Pacific Islands, Brazil and the West Indies (Traquair, 1947). On the other hand, pyomyositis is very rare in temperate climates. Abram (1904) reported two cases from which streptococci were isolated, and Barrett & Gresham (1958) also reported four fatal cases from which group A streptococci were isolated. Adams, Denny-Brown & Pearson (1962), who have not seen a single case of suppuration in voluntary muscle, remark that 'the rarety of abscess formation in muscle even in the face of overwhelming septicaemia...indicates that muscle tissue does not provide a suitable medium for the growth of many bacteria'. This statement is certainly untrue for Africans in Uganda where the voluntary muscles are one of the commonest sites of suppuration due to pyogenic bacteria. However, cases have been described from temperate climates. Clark (1887), in England, reported a fatal case but no bacteriological examination of the pus was made. Ryle (1949) described a case without comment in his essay on staphylococcal fever and seven cases were reported from Sweden in 1924 (Holm, 1924). Cases have also been reported in Europeans resident in the tropics (Robin, 1961).

Various organisms have been isolated from the pus including a Pasteurella (Bouffard, 1920) and non-haemolytic streptococci (Robin, 1961). However, by far the most frequently reported organism has been the staphylococcus, usually *Staphylococcus aureus* but sometimes *Staph. albus.*

Fleming (1930) was the first to report a case from Uganda and *Staph. aureus* was isolated from this patient. But the disease certainly occurred long ago in Uganda for in the manuscript case notes of the Mengo Hospital, Kampala, for 1897 there is a case which can be confidently recognized as pyomyositis and in another case of the following year there is a drawing of some pus under the microscope illustrating the contained staphylococci. Cook (1963) reported a declining incidence of pyomyositis from 250 cases seen at Mulago Hospital, Kampala, in 1948 to 71 in 1961. Nevertheless, the disease is still very common. The present series of 79 cases was collected in about 9 months in 1964 and many cases have certainly been missed. The object of this paper is to present a detailed study of the bacteriology of these typical cases of tropical pyomyositis and to compare the phage-types and

antibiotic sensitivity pattern of the pyomyositis staphylococci with strains isolated from other lesions and from healthy nasal carriers.

It has been suggested that pyomyositis is associated with two specific infectious diseases: leptospirosis (Meyer-May & Vaucell, 1936) and syphilis (Manson-Bahr, 1960). Serum from some of the present patients has therefore been tested for evidence of leptospirosis and treponemal infection.

THE CLINICAL MATERIAL

The pyomyositis cases consisted of 79 patients. They were quite typical, the clinical history being usually one of pain and swelling of the affected part for 1 or 2 weeks. Any of the larger muscles may be affected, the calf, thigh, buttocks, and lumbar, subscapular and pectoral regions, as well as the arm have all been affected in the present series. Not infrequently several different sites were involved. At operation large abscesses were found deep to the deep fascia.

The strains of staphylococci isolated from other lesions for comparative purposes were an unselected collection of strains isolated in the clinical laboratory from hospital in-patients with a variety of infections such as osteomyelitis, wound infections, burns, skin and chest infections.

The nasal carrier strains were isolated from an unselected population of all ages and both sexes of Ugandans at school or attending dispensaries as out-patients. They had no connexion with the in-patient hospital environment. Table 1 shows sex and age distribution by decades of the pyomyositis patients where this information was available.

Table 1. *The distribution according to sex and age (decade) of fifty-three cases of pyomyositis*

	Decade							
	1st	2nd	3rd	4th	5th	6th	7th	Total
Male	8	10	4	8	4	1	1	36
Female	3	4	7	0	2	1	0	17

METHODS

Pus was collected at operation and plated out as soon as possible on human blood agar plates and incubated overnight at 37° C. both aerobically and anaerobically. A Gram-stained film of the pus was also examined microscopically.

The organisms isolated were identified by conventional methods. *Staph. aureus* was identified on the basis of typical colonial appearance, morphology and a positive slide coagulase test using citrated rabbit plasma. The staphylococci were phage-typed at the Staphylococcus Reference Laboratory, Colindale Avenue, London.

Antibiotic sensitivity was tested on 'Oxoid' Sensitivity agar using 'Oxoid' Multodiscs containing penicillin 1·5 units, streptomycin 10 μg., tetracycline 10 μg., chloramphenicol 10 μg., erythromycin 10 μg. and novobiocin 5 μg. Serum anti-α-lysin titres were measured using the method described by Lack & Towers (1962).

The Wassermann reaction (W.R.) and the Reiter protein complement fixation (R.P.C.F.) test were done in the standard way using a 1/5 dilution of patient's serum and 3 m.h.d. complement with incubation for 1 hr. at 37° C.

RESULTS

Table 2 shows the organisms isolated from the pus of the 79 cases of tropical pyomyositis. It will be seen that in most cases *Staph. aureus* was isolated in pure culture. Table 3 shows the distribution of the pyomyositis, hospital and carrier strains amongst the three main phage groups. The percentage of the total, in round figures, is shown in parentheses. The most striking observation is that 60 % of the pyomyositis strains belong to phage Group II as compared with 22 % for

Table 2. *Organisms isolated from the pus of seventy-nine cases of pyomyositis*

Organism	No. of cases
Staph. aureus	74
Staph. aureus +Group C Strep.	1
Group A Strep.	1
Strep. viridans	1
Non-haemolytic Strep.	2

Table 3. *The distribution of pyomyositis, other lesion and nasal carrier strains of* Staphylococcus aureus *between the three phage groups (percentages in parentheses)*

	Pyomyositis	Other lesions	Carrier
Group I	13 (18)	12 (24)	18 (20)
Group II	44 (60)	11 (22)	19 (21)
Group III	16 (21)	23 (46)	31 (35)
Untypable	1 (1)	4 (8)	21 (24)
Total	74	50	89

Table 4. *Comparison of the antibiotic resistance pattern of the pyomyositis, other lesion and carrier strains of* Staphylococcus aureus *(percentages in parentheses)*

Number of antibiotics to which resistant	Pyomyositis	Other lesions	Carrier
0	15 (20)	7 (14)	60 (67)
1	43 (58)	19 (38)	20 (23)
2	15 (20)	18 (36)	7 (8)
3	1 (2)	6 (12)	2 (2)

other lesions and 21 % for the carrier strains. Of the 44 Group II pyomyositis strains 32 had a single phage-typing pattern 3A/3B/3C/55/71 or did not differ from it by more than a single phage reaction, whereas this phage pattern accounted for only eight of the 50 strains from other lesions and nine of the 89 carrier strains. Table 4 shows the number of strains in each series which were either sensitive to all antibiotics tested or resistant to one, two or three antibiotics. Generally those

strains which were resistant to a single antibiotic were penicillin resistant; when resistant to two antibiotics to penicillin and streptomycin and when resistant to three antibiotics to penicillin, streptomycin, and tetracycline but occasionally a different pattern occurred. It will be seen that although the pyomyositis strains seldom showed multiple resistance they were penicillin resistant much more often than the carrier strains in the population from which they were presumably derived.

The results of the examination of serum samples from a number of patients for agglutinins to leptospira, for anti-staphylococcal alpha-lysin and for evidence of syphilis were as follows:

Leptospiral agglutinins. Eight serum samples were tested and all showed titres of 1/10 or less to both *L. icterohaemorrhagiae* and *L. canicola.* (These tests were kindly done by Dr P. Bradstreet of the Central Public Health Laboratory, Colindale Avenue London.)

Anti-staphylococcal alpha-lysin titres. Twenty serum samples were tested; 14 showed levels of 2 units per ml. or less, four showed 4 units per ml., one showed 6 units per ml. and one showed 16 units per ml.

Serological evidence of syphilis. Twenty serum samples were tested, three gave positive reactions with the W.R. and R.P.C.F. test. A further serum gave a positive W.R. but negative R.P.C.F. test. The remaining 16 sera were negative with both tests.

DISCUSSION

The character of staphylococcal strains from pyomyositis

The most interesting point in these observations is that 60% of the pyomyositis staphylococci belong to phage Group II and that 43% possess the single phage-typing pattern 3a/3b/3c/55/71 or do not differ from it by more than a single phage-pattern reaction. This is unusual in staphylococcal infections where, in general, all phage types are capable of causing the whole range of staphylococcal disease. The only previously described well-substantiated exceptions are: (1) that 70% of staphylococci from cases of impetigo are lysed by phage 71 only; and (2) that 83% of staphylococci from outbreaks of food-poisoning belong to phage Group III (Anderson & Williams, 1956; Williams, Rippon & Dowsett, 1953). There is, however, a tendency for some phage-types of staphylococci to be associated more with certain broad groups of infections than others. For example, Williams *et al.* (1953), in England, found that of 34 strains from fulminating, post-influenzal pneumonia 60% belonged to phage Group I but this was not true of other cases of staphylococcal pneumonia. Rountree (1953), in Australia, reported that of 59 strains from deep infections such as septicaemia, osteomyelitis and abscesses 44% belonged to phage Group II. This last observation is of special interest in the present connexion because although the phage patterns of this group are not stated nor the 'deep infections' characterized in detail it was noted that 20 out of 39 staphylococci from 'abscesses' belonged to phage Group II. We do not know the situation of these abscesses but they are distinguished from the group of boils and carbuncles. Parker (1958) also found a higher proportion of Group II strains in deep lesions as compared with superficial lesions. Williams & Jevons (1961)

showed that 31·3 % of staphylococcal infections arising outside hospital were caused by Group II strains, whereas Group II strains were responsible for only 11·1 % of infections acquired in hospital.

If all strains of staphylococci were similarly endowed with respect to pathogenicity one would expect the frequency of infecting types to be proportional to the frequency with which types were carried by the healthy population. This might be true in an uncomplicated situation consisting of cases of staphylococcal infection arising in an antibiotic-free environment. But since some strains are more capable of developing antibiotic resistance than others these strains have for a long time been selected to form 'hospital strains' and the same thing is true to a lesser extent outside hospitals. Of the 3a/3b/3c/55/71 strains causing pyomyositis 26 out of 32 were resistant to penicillin as compared with only two out of 10 carrier Group II strains and four out of 10 hospital Group II strains. It is true that many of the pyomyositis patients had been treated with penicillin before coming into hospital, but the onset of pyomyositis is rapid and it seems unlikely that the staphylococci could become resistant during the brief period of therapy. Moreover, of the other strains besides Group II strains isolated from pyomyositis only 14 out of 29 were penicillin resistant, despite the fact that they belonged to phage groups which acquire antibiotic resistance more rapidly. This suggests that staphylococci with the phage pattern 3a/3b/3c/55/71 and which are penicillin-resistant are most characteristic of pyomyositis.

Associated aetiological factors in pyomyositis

At one time or another a number of factors have been claimed to be associated with or to precipitate pyomyositis but none have been well substantiated. Burkitt (1947) investigated the possible association of pyomyositis with filariasis, ankylostomiasis, malaria and sickle-cell anaemia with negative results. Ashken & Cotton (1963) have suggested that subclinical scurvy and trauma may be predisposing causes. These factors have not been investigated in the present series.

Meyer-May & Vaucel (1936), working in Hanoi, found that 12 out of 54 cases of pyomyositis showed agglutinin titres of between 1/300 and 1/1000 against *Leptospira icterohaemorrhagiae*. Sera from the first eight patients in the present series were tested for leptospiral agglutinins and all found to be negative.

Manson-Bahr (1960) stated that 50 % of cases of pyomyositis have a positive Wassermann reaction and recommended anti-syphilitic therapy. The serum of 20 patients in the present series was tested and three were found to have both a positive W.R. and R.P.C.F. test and should therefore be regarded as having evidence of previous treponemal infection. A fourth patient with a positive W.R. but a negative R.P.C.F. test should probably be regarded as a false positive not indicating treponemal infection. A difficulty in associating any condition with treponemal infection in the tropics is that serological evidence of such infection may be found in a high proportion of the population. If reliance is placed on the W.R. alone positive reactions will be found not only due to true treponemal infections but also to false positive reactions due to such conditions as malaria. There are no published figures showing the incidence of serological evidence of syphilis in Uganda but a

comparison with a small number of sera recently tested suggests that three out of 20 positive is not in excess of the rate found in the general population.

The investigation here reported shows that staphylococci of phage Group II are more frequently found in cases of pyomyositis occurring in Uganda than would be expected from their incidence amongst healthy nasal carriers or other types of staphylococcal infection. Why this should be so is unknown but it is in line with the observations of Williams & Jevons (1961) that a higher proportion of Group II strains are found amongst staphylococcal infections arising outside hospital than amongst nasal carriers or staphylococcal infections arising in hospitals. As Dr M. T. Parker has suggested to me it may be that Group II strains are particularly well able to penetrate unbroken skin and cause infections. It would be of interest to know the phage-types of staphylococci found in pyomyositis in other parts of the world. However, factors other than a particular type of staphylococcus are clearly important in the aetiology of pyomyositis since many strains of staphylococci as well as other organisms can cause the disease. There may be some impairment of the patient's immune response, for 14 out of 20 of the present series showed a serum anti-alpha-lysin titre of less than 2 units per ml. on a single sample of serum taken about the time of operation. This may, however, be merely a reflexion of the fact that the disease up to operation is usually short. It was not possible to take serial blood samples.

The source of the infecting staphylococcus is not known. Although it has been claimed that there is sometimes a history of recent boils this does not seem to be usual. The carrier state of the patients before operation would be of considerable interest. It has been possible to test this in the present series in only three cases, in which nasal and perineal swabs showed no staphylococci. The preponderance of males amongst cases of pyomyositis which has been found in the present series may be of some significance, but might be due to a general difference in admission rate between the two sexes. More males than females are generally admitted to the New Mulago Hospital. However, all authors who have drawn their cases from the general population, rather than special groups such as soldiers, have noted this male preponderance, although Cook (1963) reported a narrowing of the sex difference from 5·5:1 between 1948 and 1955 to 2·7:1 between 1957 and 1961. If males are more susceptible than females it would seem likely that this is innate rather than environmental since the preponderance is found even in the first decade of life when environmental factors must be very similar for both sexes. Nor does it seem likely that trauma is a significant aetiological factor for it is doubtful if, in Uganda, males are subject to more trauma than females and the disease occurs in infants but a few months old.

A comparison of Uganda staphylococci with those from other parts of the world

I am not aware of any reports of the frequency of different phage-types in Uganda so that these results have some interest as showing, although the numbers are small, something of the distribution of different phage-types which can be compared with findings in other parts of the world, such as the data of Williams *et al.* and Parker from England and Rountree from Australia. Table 5 compares the

percentage of nasal carrier strains falling into three main phage groups. Table 6 compares 140 hospital strains tested by Williams *et al.* (1953), 76 strains from wounds and burns tested by Rountree (1953) and 78 strains from deep infections and 91 strains from superficial infections tested by Parker (1958) with the non-pyomyositis strains isolated in Uganda. The difference is not striking, particularly if one allows for the difference in the year in which the samples were collected and differences in availability of antibiotics in different countries which would be expected to favour the survival of Group III strains.

Table 5. *The distribution of nasal carrier strains of* Staphylococcus aureus *between the three phage groups in Uganda compared with England and Australia*

	Percentage of strains in		
	Group I	Group II	Group III
Williams *et al.* (1953)	24	14	19
Parker (1958)	34	22	15
Rountree (1953)	19	20	27
Uganda	20	21	34

Table 6. *The distribution of the non-pyomyositis hospital strains of* Staphylococcus aureus *between the three phage groups compared with strains isolated from lesions in England and Australia*

	Percentage of strains in		
	Group I	Group II	Group III
Williams *et al.*	14	4	56
Parker (deep)	33	36	13
Parker (superficial)	21	20	31
Rountree	20	21	24
Uganda	24	22	46

SUMMARY

1. The bacteriology of the pus from 79 cases of tropical pyomyositis has been studied. In 74 cases *Staphylococcus aureus* was isolated in pure culture.

2. Phage typing showed that 60 % of the pyomyositis strains belonged to phage Group II as compared with 22 % for miscellaneous hospital infections and 21 % for nasal carrier strains.

3. A high proportion of the Group II strains from pyomyositis were found to be penicillin resistant and it is thought that this is unlikely to be due to the use of penicillin therapeutically but is probably a natural characteristic of the strains.

4. Other suggested aetiological factors in pyomyositis such as leptospirosis and syphilis have been investigated but no association has been found.

5. The phage types of staphylococci found in Uganda have been compared with those reported from England and Australia and found not to be strikingly different.

I wish to thank Dr M. T. Parker and Dr M. P. Jevons not only for phage-typing staphylococci but for their helpful comments and suggestions.

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J. Hyg., Camb. (1965), **63**, 525
Printed in Great Britain

The antibody response of rabbits to inactivated vaccinia virus

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(*Received* 7 *July* 1965)

INTRODUCTION

The often unpleasant and occasionally serious side effects of Jennerian vaccination have stimulated many studies on the antigenicity of inactivated vaccinia virus, with the object of using inactive preparations either as a substitute for live virus, or to provide a basic immunity which might protect against the illness of subsequent vaccination with live virus without interfering with its effectiveness.

Most investigations have been directed to the problem of immunogenicity of inactivated vaccinia virus, but the situation has not been clarified. For example, Parker & Rivers (1936) demonstrated some immunogenicity of formalin-inactivated vaccine in rabbits, but considered that this was weak and unlikely to be useful for the protection of humans against smallpox. More recently, Amies (1961) demonstrated what he considered to be a negligible immune response in rabbits given formalinized vaccine with adjuvant. In contrast RamanaRao (1962) reported that formalin-inactivated vaccine with adjuvant stimulated levels of virus neutralizing antibody in rabbits approaching those resulting from dermal infection with live virus. Previously Collier, McClean & Vallet (1955) had shown that an ultraviolet (UV) inactivated vaccine without adjuvant stimulated a regular immune response in rabbits. A similar vaccine (five times concentrated) tested in man was immunogenic in only about 50 % of subjects (Kaplan, McClean & Vallet, 1962). Kaplan (1962) extended the study of this UV-inactivated vaccine in humans and showed that 50 times the rabbit dose stimulated substantial increase in the level of virus neutralizing antibody in individuals who had previously been vaccinated with live vaccine.

There is no doubt therefore that inactivated vaccinia virus can be immunogenic. Precise evaluation of the conditions necessary to ensure immunogenicity is difficult, and this is largely due to the variety of methods and criteria of immunogenicity which have been used by different workers.

Several conditions could be of critical importance. The dose of virus antigen would seem to be most obvious, and this was stressed in some of the earlier work (Bernkopf & Kligler, 1937). However, with vaccines of apparently similar virus content, e.g. that reported by Collier *et al.* (1955) and that of Amies (1961), the former was satisfactory while the latter possessed poor immunogenicity. It is obvious that the inactivating agent could be of great importance, but again the effect is not clear since Kaplan (1960) has shown that even with the same dose of virus and the same inactivating agent (gamma radiation) one vaccine can be immunogenic, while another with a longer period of inactivation is not. The

duration of contact between virus and inactivant could therefore be extremely critical, whilst other conditions, e.g. the route of immunization, may obviously play an important part.

The work reported in this paper was an attempt to clarify the effect of these factors on the immunogenicity of inactivated vaccinia virus, measured by the neutralizing antibody response in rabbits, by studying them under the same experimental conditions, so that a comparison of their importance could be made.

Four series of experiments were performed with inactivated vaccinia vaccines:

(I) Vaccines with constant virus content but with differing methods and extent of inactivation.

(II) Vaccines with differing virus content and constant method and extent of inactivation.

(III) Vaccines as in series (II) but with virus suspended in polyvinylpyrrolidone (PVP) to assess the adjuvant effect of this substance.

(IV) Vaccines administered by different routes.

MATERIALS AND METHODS

Virus. The Lister Institute strain of rabbit-adapted vaccinia virus was used. It was propagated on the skin of rabbits by the method of Hoagland, Smadel & Rivers (1940).

Rabbits. Young adult New Zealand Whites, of both sexes, weighing 2–3 kg.

Infectivity titrations

Virus was titrated by plaque counting in monolayers of HEp_2 cells. Cell cultures were made in 12 oz. flat medicine bottles at 37° C. The growth medium was Eagle's basic medium modified to contain twice the concentration of amino acids and vitamins, and containing 10 % tryptose phosphate broth (Difco) and 10 % calf serum. The confluent monolayer of cells in a bottle was suspended in 75 ml. growth medium after 2 min. treatment with 5 ml. of a solution of 0·25 % trypsin and 0·02 % 'versene' at 37° C. One-ounce bottles were used for plaque counts; they were inoculated with 5 ml. of this cell suspension which after 28 hours at 37° C. had formed an almost complete monolayer; they were then ready for virus inoculation.

Virus dilutions were made in McIlvaine's buffer of 0·004 M, pH 7·2. Growth medium was poured off and the plaque bottles inoculated with 0·5 ml. of virus. Serum-free medium was added to bring the total volume of fluid in the bottle to 2·5 ml. The bottles were placed at 37° C. overnight for virus adsorption. The fluid was then poured off and the monolayers fed with 5 ml. fluid overlay medium (modified Eagle's medium containing 10 % tryptose phosphate broth, 1 % calf serum, antivaccinia serum, and additional bicarbonate buffer). The bottles were replaced at 37° C. for a further 2 days to allow plaque development. The medium was discarded and the monolayers stained with Ziehl-Neelsen's carbol fuchsin diluted 1/20. Two or three bottles were used for each dilution of virus.

Fluid overlay containing antiserum was used instead of a solid overlay to

prevent formation of secondary plaques. Antiserum can be used for this purpose since it is known that vaccinia virus spreads directly from cell to cell in a monolayer (Nishmi & Keller, 1962). This method has two important limitations: (i) virus released into the fluid medium may not be completely neutralized even when the antibody is present in excess, and (ii) infected cells may become detached and initiate secondary plaques by direct cell-to-cell spread. The antiserum overlay was therefore used only to gain an additional 24–48 hr. in plaque development time. The antiserum used was antivaccinia serum prepared in rabbits 3 weeks after they had recovered from a vaccinia infection over a wide area of skin by giving them three intravenous inoculations, at weekly intervals, of a clarified extract of rabbit-skin virus. The animals were bled out 1 week after the final inoculation and the sera pooled, sterilized by Seitz-filtration, and stored at $-20°$ C. The antiserum was used in the overlay medium at a dilution which prevented the appearance of secondary plaques for at least 24 hr. after the time at which the plaques were normally counted.

Preparation of virus suspensions

Virus from infected rabbit skin was purified by one cycle of differential centrifugation, followed by centrifugation in a sucrose density gradient using the method of Zwartouw, Westwood & Appleyard (1962). Sucrose was removed from the virus suspension by dialysis against two changes each of 1000 vol. of McIlvaine's buffer 0·004 M, pH 7·2, for a total of 6 hr. at 4° C.

Preparation of vaccines

(*a*) *Constant virus content, variable inactivant and extent of inactivation.* Sixteen vaccines were prepared from a pool of active virus, of which 15 fell into three groups according to the method of inactivation—hydroxylamine, formalin or heat. *Hydroxylamine* is thought to react exclusively with nucleic acid (Lie, 1964). It was used in these experiments as a convenient method of inactivating virus with minimal interference with protein antigens. A solution of 2×10^{-3} molar was made up immediately before use. This was mixed with an equal volume of purified virus and inactivation took place in the dark at room temperature. *Formalin* in low concentrations was used to inactivate virus by a relatively mild effect upon protein antigens. The method used was that of Gard (1957). Purified virus was mixed with an equal volume of 0·12 M formalin, 0·04 M glycine and allowed to react in the dark at room temperature. *Heat* inactivation was used to cause more damage to viral protein antigens than the others and was achieved by placing tubes containing purified virus in a water bath at 50° C. Preliminary inactivation curves were obtained in order to determine suitable inactivation times for the production of vaccines. For each method of inactivation five vaccines were prepared at stages during the course of inactivation. Virus was removed from the inactivant by centrifugation in a Spinco L ultracentrifuge at 30,000 rev./min. for 30 min. The virus 'pellet' was resuspended by ultrasonic vibration in McIlvaine's buffer 0·004 M, pH 7·2, containing 10% polyvinylpyrrolidone.* This substance was used

* 'Plasdone C'; supplied by Fine Dyestuffs and Chemicals Ltd., Manchester.

because of its stablizing effect on virus infectivity and adjuvant effect (Amies, 1962). In order to preserve uniformity the heat inactivated virus was treated exactly as the hydroxylamine and formalin inactivated virus. Table 1 shows the residual virus infectivity (pfu/ml.) of each of these vaccines.

Table 1. *Method and extent of inactivation for each vaccine in the first series*

	Vaccine	Temp.	Titre (pfu/ml.)
1	Active	—	2×10^7
2	Formalin 12 hr.	19° C.	6×10^3
3	Formalin 36 hr.	19° C.	3×10^1
4	Formalin 48 hr.	19° C.	Nil
5	Formalin 96 hr.	19° C.	Nil
6	Formalin 192 hr.	19° C.	Nil
7	Hydroxylamine 3 hr.	19	$1{\cdot}6 \times 10^3$
8	Hydroxylamine 5 hr.	19	10
9	Hydroxylamine 7 hr.	19	2
10	Hydroxylamine 16 hr.	19	Nil
11	Hydroxylamine 48 hr.	19	Nil
12	Heat 30 min.	50	$6{\cdot}4 \times 10^2$
13	Heat 2 hr.	50	10
14	Heat 4 hr.	50	2
15	Heat 7 hr.	50	Nil
16	Heat 24 hr.	50	Nil

Table 2. *Estimated virus content of inactivated vaccines based on infectivity before inactivation*

Vaccine	Virus content pfu/dose (0·5 ml.)
17	$2{\cdot}8 \times 10^8$
18	7×10^7
19	$1{\cdot}8 \times 10^7$
20	4×10^6

(*b*) *Variable virus content vaccines in buffer.* Four formalin inactivated vaccines were prepared by mixing equal volumes of purified virus with 0·012 M formalin, 0·04 M glycine, and allowing inactivation to take place in the dark at room temperature for 100 hours. Virus was removed from the formalin by centrifugation at 30,000 rev./min. for 30 min. in the ultracentrifuge, and the virus 'pellet' resuspended in McIlvaine's buffer 0·004 M, pH 7·2 by ultrasonic vibration. Allowing for losses during centrifugation the approximate virus content of each vaccine was as shown in Table 2.

Each vaccine was tested for residual infectivity by inoculation onto the chorioallantoic membranes of 12-day-old chick embryos, using 4 eggs per vaccine and 0·1 ml. of inoculum. Active virus was not detected in any of the vaccines.

(*c*) *Variable virus content vaccines in PVP.* Three similarly prepared formalin-inactivated vaccines were suspended in McIlvaine's buffer pH 7·2 containing

10 % PVP. The approximate virus content of each of these vaccines is shown in Table 3.

Infectivity testing of these vaccines on the chorioallantoic membrane did not reveal any active virus. All vaccines were dispensed in 0·5 ml. amounts in sealed ampoules and stored at −70° C.

Table 3. *Estimated virus content of inactivated vaccines containing polyvinylpyrrolidone, based on infectivity before inactivation*

Vaccine	Virus content pfu/dose (0·5 ml.)
21	9×10^7
22	3×10^7
23	6×10^6

Immunogenicity testing

The immunogenicity of the vaccines was assessed by measurement of virus neutralizing antibody produced by rabbits following two intramuscular inoculations of 0·5 ml. of vaccine. In series (I) (variable inactivation) there was an interval of 4 weeks between inoculations: blood was obtained before and 2 weeks after each inoculation. In the second, third and fourth series the inoculations were given 6 weeks apart and the animals bled before inoculation, and at 5, 10, 20, 30 and 42 days after this. They were bled again 14 days after the second inoculation. Each individual vaccine was tested in four rabbits.

Measurement of neutralizing antibody

Appropriate twofold dilutions of serum were made in dilute McIlvaine's buffer 0·004 M, pH 7·2, in the unit volume of 0·3 ml. An equal volume of test virus was added and the mixture incubated for 2 hr. at 37° C. Monolayers of HEp_2 cells in 1 oz. plaque bottles were inoculated with 0·2 ml. virus–serum mixture using two bottles for each serum dilution. After absorption of unneutralized virus the monolayers were overlaid and incubated as described for plaque assay. A virus control consisting of 1 vol. of test virus and one volume of buffer was included in each batch of tests. The control mixture was incubated for 2 hr. at 37° C. and 0·2 ml. volumes inoculated into each of four plaque bottles. The average plaque count in the control bottles was taken as 100 % virus survival and the titre of a serum was expressed as the reciprocal of the dilution of serum which allowed 50 % virus survival. The test virus suspension was vaccinia virus grown in HEp_2 cells and partially purified by differential centrifugation. This virus was suspended in 20 % skim milk in dilute McIlvaine's buffer and stored in 1 ml. amounts at −70° C. For each batch of tests one of these bottles was thawed and further diluted in 20 % skim milk in dilute McIlvaine's buffer to give a virus control plaque count of approximately 100. Skim milk was used in the suspending medium for virus in the neutralization test in order to prevent heat inactivation of the virus during incubation of virus serum mixtures (Boulter, 1957).

RESULTS*

First series of experiments

The most striking feature of these results was the great variation in the titres of antibody obtained even within a group of rabbits receiving the same vaccine. Figure 1 shows the levels of antibody for each rabbit 2 weeks after the first dose of vaccine, and also the residual virus infectivity (pfu/ml.) in each vaccine. Most rabbits had developed negligible quantities of antibody ($< 1/10$), but a few, especially those receiving hydroxylamine inactivated vaccines, developed antibody to a much higher titre.

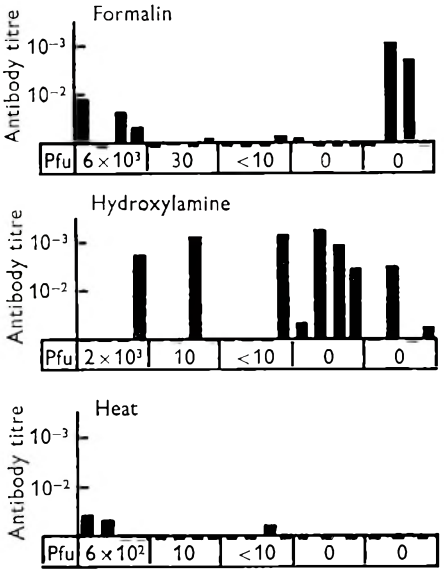

Fig. 1. Neutralizing antibody titres in rabbits 2 weeks after a single inoculation of formalin, hydroxylamine or heat inactivated vaccines. Residual virus infectivity of each vaccine is given in pfu/ml.

Second series of experiments

A possible explanation for the variable results obtained in the first series was that the vaccines contained antigen within the critical concentration range, resulting in a poor and transient antibody response in some rabbits and a good and more enduring response in others (Svehag & Mandel, 1964). This series was undertaken to determine whether or not this type of variable antibody response occurred with inactivated vaccinia virus, and within what range of virus concentrations. The antibody responses obtained were of two types: (i) a poor response characterized by a low titre maximal 5 days after inoculation which declined to undetectable levels by 10–20 days, and (ii) a good response characterized by a higher titre maximal 10 days after inoculation, antibody being detectable 6 weeks after inoculation.

* Complete tables of results are available in the author's M.D. thesis, The Queen's University of Belfast.

Third series of experiments

The maximum antibody titres obtained after one inoculation in good responses in the second series (1/190) were much lower than the highest titres developed by some rabbits in the first series (1/2000). This difference might have been due to an adjuvant effect of the PVP contained in the vaccines of the first series. To clarify this and to evaluate the adjuvant property of PVP the third series of experiments was undertaken. The results again showed the two types of antibody response, but neither the incidence of good responses in relation to the concentration of virus in the vaccines nor the antibody titres obtained were any higher than those achieved with similar vaccines which did not contain PVP.

Fourth series of experiments

A comparison was made of the antibody response to vaccine 17 (Table 2) when given intradermally (I.D.) or subcutaneously (S.C.) and that following intramuscular (I.M.) inoculation. All inoculations (0·5 ml.) were made into the left thigh. The intradermal vaccine was divided into 5 inocula of 0·1 ml. and these were all

Table 4. *Geometric mean of neutralizing antibody titres 2 weeks after the second dose of vaccine 17 when given by different routes*

Route	Geometric mean titre
I.M.	887
S.C.	5175
I.D.	5549

given into as small an area of skin as was possible. The high dose vaccine was used in the expectation that all rabbits would show the good type of antibody response, and therefore make a more valid comparison of antibody titres between the groups. With the exception of one rabbit in the S.C. group all rabbits showed the good type of antibody response. The antibody titres 2 weeks after the second inoculation indicated that the most uniform response followed I.D. inoculation. Titres following I.M. inoculation were much lower, and those following S.C. inoculation were much more variable, although if the rabbit which showed the poor transient response is excluded, the geometric mean titre of antibody after two inoculations of vaccine was similar to that following I.D. inoculation. Geometric mean titres for each group are shown in Table 4.

Summary of antibody responses in relation to dose of antigen

The small number of rabbits which received each individual vaccine makes it impossible to evaluate each vaccine precisely. The combination of data from the second, third and fourth series of experiments clearly shows the importance of the quantity of antigen in determining the immunogenicity of these vaccines. Table 5 shows the relationship between the antigen content of each vaccine, based on pfu of infectivity before inactivation, and the incidence of good antibody responses in each group of vaccines. This also shows that variable antibody responses are associated with the virus concentration range 10^7–10^8.

Table 5. *Relationship between concentration of virus (pfu/0·5 ml. before inactivation) and incidence of good antibody responses*

Virus content of vaccine (pfu/0·5 ml.)	Incidence of good antibody responses
$2{\cdot}8 \times 10^8$	11/12
9×10^7	2/3
7×10^7	3/4
3×10^7	2/4
$1{\cdot}8 \times 10^7$	1/4
6×10^6	0/4
4×10^6	0/4

DISCUSSION

Rather than helping to clarify the position of the inactivating agent and the extent of inactivation in the immunogenicity of inactivated vaccinia virus, the results of the first series of experiments seem to add further confusion to the problem. Most of the rabbits developed low titres of antibody, but a few developed a much higher titre, especially in the hydroxylamine-inactivated vaccine series. The occurrence of these high reactors did not seem to bear any relationship to the degree of inactivation, and even within a group of rabbits receiving the same vaccine some developed high titres of antibody and others very low titres. The most obvious explanation is that the higher titres resulted from infection with live virus either by incomplete inactivation, by some reactivation process, or by accidental infection. But these possibilities seem unlikely since the last two vaccines in each series were devoid of infectivity for either the chorioallantoic membrane or for tissue culture, and even when a partially inactivated vaccine with a residual infectivity of approximately 10^3 pfu was inoculated, not all rabbits showed the high antibody response. Accidental dermal infection at the site of inoculation was not seen in any of the rabbits, and in order to minimize the risk of accidental infection by other means the rabbits which received totally inactivated vaccines were kept in a separate building. Furthermore none of the preimmunization sera from these rabbits showed detectable antibody activity. If these results are not easily explicable in terms of infection with live virus, is there any alternative explanation?

Svehag & Mandel (1964), using poliovirus as antigen, have shown that in rabbits there can be variable antibody responses to the same quantity of antigen, provided the quantity of antigen is within a certain range. Below this range all rabbits showed a low and transitory response and above it all rabbits showed a higher and more enduring response. Within the critical range some rabbits gave the low and others the high response, the type of response being dependent upon the individual rabbit. Assuming that the quantity of antigen was within this critical range a similar phenomenon with inactivated vaccinia virus could explain the results of the first series of experiments, and also explain the variability in

effectiveness of inactivated vaccinia virus vaccines which has been such a feature of the literature.

The results of the second and third series of experiments show (i) that this variable response occurs with inactivated vaccinia virus vaccines when the quantity of antigen corresponds to an infectivity titre of approximately 10^7 to 10^8 pfu before inactivation, but (ii) that the titres obtained in good antibody responses were still lower than those high titres obtained in the first series, and (iii) that this was not due to any adjuvant effect of the PVP in the first series of vaccines.

It is difficult to avoid the conclusion that the animals showing the high titres of antibody in the first series had become accidentally infected with vaccinia virus at some time between the taking of the preimmunization serum and the first dose of vaccine although for the stated reasons it is difficult to see how this could have happened. That accidental infection of rabbits with vaccinia virus can easily occur, in spite of precautions taken to prevent it, has been pointed out by Parker & Rivers (1936). Whatever the explanation for these high titres may be, it is clear that a single antibody measurement taken at an arbitrarily chosen time within 2–3 weeks after administration of antigen can involve difficulties in interpretation. If the dose of antigen is within the critical range where variable antibody responses may occur, and this dose is varied unintentionally, even within small limits, the calculation of mean antibody titres in relation to some other factor may lead to erroneous conclusions. The experiments on the route of immunization indicate that the highest and most uniform antibody response was obtained by intradermal inoculation, assuming that the division of the intradermal doses was unimportant. Antibody responses following subcutaneous inoculation were very variable and one animal in this group showed a poor response to the first dose of vaccine. Whether this was an immunologically refractory rabbit or whether it indicated a lesser efficiency of the subcutaneous route is a problem which would obviously require to be studied in a much larger number of animals.

In conclusion, the dose of virus is the most important single factor in determining the immunogenicity of inactivated vaccinia virus vaccines when the immunogenicity is assessed by the development of virus neutralizing antibody. If the high antibody titres found in the first series of experiments can be discounted on the ground that the rabbits may have been accidentally infected with live virus, it can be concluded that neither the method nor the degree of inactivation within the limits tested plays a major part in determining immunogenicity. The intradermal route of immunization would seem to have the advantage of providing both a uniform and a high level of antibody production compared with either the intramuscular or subcutaneous routes.

If an inactivated vaccinia virus vaccine of assured immunogenicity can be produced there are many problems in its practical application. It has been shown by Beunders, Driessen & van den Hoek (1960) that primary immunization with inactivated vaccine confers considerable protection against the illness of subsequent Jennerian vaccination. Herrlich (1964) has reported that primary immunization with inactivated vaccine results in a lower incidence of post-vaccinal

encephalitis following subsequent vaccination, though lack of control groups in this study must limit its value.

Even if it can be shown conclusively that primary immunization with inactivated vaccine can protect against most of the complications of live virus vaccination, it would be of great importance to determine the immunization schedule which would strike a balance between maximal protection from the vaccination illness, and minimal interference with the effectiveness of vaccination. It would be undesirable to induce a degree of skin immunity with the inactivated vaccine which would make it more difficult to get a subsequent vaccination 'take'. Bearing this in mind it would be interesting to discover whether or not a rapid though transient antibody response to inactivated vaccine would be sufficient to protect against the 'illness of vaccination' if live and inactivated vaccine were given simultaneously. It is probable, however, that many of those who develop vaccinia gangrenosa or generalized vaccinia have immunological defects, and it is unlikely that primary immunization with inactivated vaccine will benefit such people.

The major problem in using an inactivated vaccine as a substitute for Jennerian vaccination is to show that it can be effective. The one indubitable fact about Jennerian vaccination is that it protects against smallpox. The mechanism of this protection is, however, poorly understood. No generally acceptable laboratory criteria are therefore available by which the results of any major change in the method of vaccination can be assessed, be it the use of a further-attenuated live virus, an inactivated vaccine, primary immunization with inactivated vaccine or the simultaneous use of gamma globulin or an anti-viral drug. If sound laboratory criteria for the assessment of immunity to smallpox were available, this, in conjunction with the epidemiological data available, would provide a firm basis for the formation of scientifically acceptable vaccination policies.

SUMMARY

Inactivated vaccinia virus vaccines were prepared from purified virus inactivated by either formalin, hydroxylamine or heat. The immunogenicity of these vaccines was assessed in rabbits by measurement of virus neutralizing antibody following each of two inoculations. It was concluded that inactivated vaccinia virus stimulates the production of neutralizing antibody and that the most important single factor in this immunogenicity is the concentration of virus in the vaccine. Vaccines prepared from virus suspensions containing 10^7 to 10^8 pfu/ml. before inactivation give variable antibody responses.

I wish to thank the National Fund for Research into Poliomyelitis and Other Crippling Diseases for their support in the form of a Research Fellowship during the tenure of which this investigation was made.

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J. Hyg., Camb. (1965), **63**, 537
Printed in Great Britain

The Zermatt typhoid outbreak in 1963*

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(*Received* 9 *August* 1965)

INTRODUCTION

The major causes of typhoid outbreaks in the past have been food and milk contaminated by typhoid carriers, and water supplies also contaminated by carriers either directly or by admixture with sewage. Of these vehicles of infection water was probably much the most important. With the improvements in sanitation—both in sewage disposal and maintenance of the purity of water supplies—the incidence of waterborne outbreaks of typhoid fever has fallen sharply in the most advanced countries.

One of the most important waterborne outbreaks studied in the past 30 years was that in Croydon, England, in 1937 (Holden, 1939; Report, 1938). There were 310 cases with 43 deaths. This outbreak was due to contamination of a well by a workman who was a typhoid carrier. A waterborne outbreak occurred in Glion, Switzerland, in 1945 (Herter, 1947). There were 101 cases with 16 deaths. In both the above outbreaks the case fatality rate was high by present-day standards, 13·9 % in Croydon and 15·8 % in Glion. The introduction of chloramphenicol has dramatically changed the prognosis, the case fatality rate having now been reduced to the neighbourhood of 1 %.

The occurrence of a typhoid outbreak in an international holiday centre always presents special problems, but nowadays these are still further complicated by the rapid distribution of incubating cases, unaware of their infection, returning to their native countries by air. The tracing of the full extent of such an outbreak may thus be a very difficult task (see Anderson, 1964).

The present report is concerned with such an outbreak. This occurred in Zermatt during the winter season of 1963. There were 437 identified cases with a total of three deaths (0·7 %). The population at risk was estimated to be about 10,000 and the epidemic occurred at the peak of the tourist season. There were 260 tourists and 177 local inhabitants affected. The outbreak was due to *Salmonella typhi* Vi-phage-type E_1 (Beer, 1963); this was confirmed in ten laboratories of six countries whose nationals were infected. It was possible to estimate the probable period of infection because of information provided by the 260 infected tourists. The concomitant occurrence of an outbreak of influenza obscured the onset of

* The preparation of this report was made possible by the Service de la Santé Publique du Canton du Valais, and by a grant from the Fonds National Suisse de la Recherche Scientifique.

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the typhoid epidemic, so that the recognition of typhoid was delayed more than 2 weeks after the end of the period of infection (see Fig. 1). As will be shown, the outbreak was most probably waterborne.

DESCRIPTION OF THE EPIDEMIC

The outbreak occurred at Zermatt, in the Canton of Valais, one of the most famous holiday resorts of Switzerland, situated at 5300 ft. (1620 m.) at the southern end of a high valley of the Alps. The village has been well known to tourists and mountaineers for the last century, because of surrounding peaks such as the Matterhorn, Monte Rosa and Gornergrat.

Table 1. *Distribution of tourist population of Zermatt and attack rate of typhoid fever according to nationality*

Winter season...	1961/62	1962/63	February 1963 (= month of infection)				
	Guest-nights				Cases		Rate/ 1000
Nation	%	%	No.	%	No.	%	g.-n.
Switzerland	33·1	36·8	31,602	33·4	77*	29·6	2·44
Germany	22·9	20·95	20,031	21·2	33	12·7	1·65
France	12	10·9	13,153	13·9	37	14·2	2·81
Great Britain	11	10·1	11,105	11·8	78	30	7·02
U.S.A.	8·95	8·6	7,408	7·8	14	5·4	1·89
Other nations	12·05	12·65	11,255	11·9	21	8·1	1·87
Total	100 (724,197)	100 (505,683)	94,554	100 (94,554)	260†	100 (260)	2·75‡

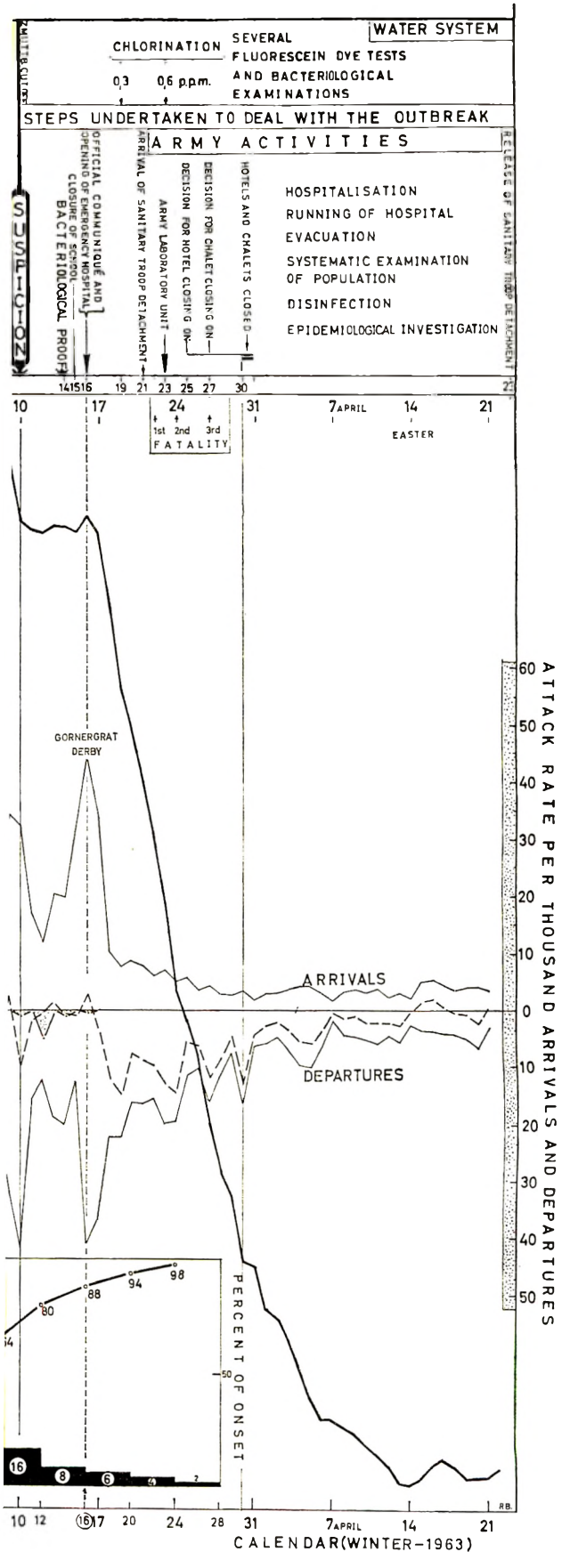
* Besides these 77 Swiss tourists Zermatt had another 177 cases of infected inhabitants and seasonal employees bringing the total victims (†) to 437 cases.

‡ Mean attack rate if British cases are excluded = 2·18 per thousand.

g.-n. = guest-nights.

The village, with a basic population of about 2200 people, has two tourist seasons, one in winter, the other in summer. During these seasons its population rises to over 10,000 people. It is accessible only by a mountain railway which collects tourists from Brig, a town 3090 ft. (940 m.) lower and 27·4 miles (44 km.) from Zermatt. Because Zermatt was accessible only by rail, it was possible to calculate the exact number of persons entering and leaving Zermatt each day. This was of great value in the quantitative examination of the epidemic.

The Zermatt Municipality analyses its tourist seasons in terms of 'guest-nights'; that is to say, each visiting nationality provides a quantum consisting of the number of persons multiplied by the total number of nights spent in Zermatt. During the winter season 1961/62 each tourist spent a mean of 9·1 nights in Zermatt. The availability of these figures made it possible to estimate the proportional attack rates of typhoid fever in the various nationalities (see Table 1). This table shows that the proportionate national representation remains roughly constant from year to year. Although the attack rate in some nationalities corresponds roughly to their guest-night percentage, there is an obvious disproportion



matt typhoid outbreak.

(*Facing p.* 539)

in other nationalities. The most pronounced of these was in the case of Great Britain, of which the guest-night percentage was only 11·8, but which provided 30% of the tourist cases. In fact, Great Britain with 78 cases had the highest number of tourists affected. It can also be seen that the outbreak resulted in the loss of over 200,000 guest-nights.

On 10 March, one of the three local medical practitioners telephoned the Mayor of Zermatt announcing the existence of a suspected case of typhoid fever. The diagnosis was based on a report from a laboratory in Sion to which the patient's serum had been sent. At the same time this practitioner notified the Health Authorities of the Canton of Valais that he suspected that a typhoid epidemic was developing. Members of the Zermatt Local Authority met immediately and decided to ask the Canton Health Authority for help. They also arranged for sampling the water supply, for the closing of one of the branches of the water supply and for a warning on the subject of the outbreak to be issued to the local practitioners.

One of the practitioners reported to the Mayor that a miner from Southern Italy who was working on a hydro-electric project higher up the valley had been admitted to his clinic with an undiagnosed pyrexia on 20 February, and had been subsequently transferred to a hospital in Brig. In retrospect, he thought that this might have been a case of typhoid fever.

A considerable number of tourists and inhabitants suffered from gastro-enteritis as early as January, and an outbreak of influenza had occurred simultaneously. At that time a hotel manager advised his guests not to drink ordinary tap water as he had received complaints of gastro-intestinal upset which, because of its association with the village, had come to be known as 'Zermatitis'. On 13 March a physician in the village reported that he had over 50 patients, inhabitants and tourists, who had fallen ill since the beginning of the month. Although these patients suffered from headache and gastro-intestinal disorder, he did not at first think that their symptoms were indicative of typhoid fever. Suspicion then arose that some patients who had been treated for influenza might have been suffering from typhoid fever.

Assistance with the investigation was immediately provided by the Public Health Laboratory of the Canton of Geneva. Samples of blood and excreta from suspected cases were also sent from Zermatt to other laboratories in Switzerland. By 13 March, the sera of several patients yielded results suggesting infection with *S. typhi* and on 14 March *S. typhi* was identified from one specimen of faeces.

The first confirmed case in England of typhoid infection from Zermatt was reported on 9 March. The culture of this case was identified as phage-type E_1 by 12 March in the Enteric Reference Laboratory at Colindale, London. All cultures isolated subsequently in this outbreak belonged to this type. Because of reports of other cases in various parts of England, the Ministry of Health issued a general alert on the subject to Medical Officers of Health on 13 March. The Director of the Enteric Reference Laboratory at Colindale sent a letter on 14 March to the Director of the Swiss Centre for Enteric Phage Typing drawing his attention to a probable typhoid epidemic, apparently waterborne, originating in Zermatt.

SUBSEQUENT DEVELOPMENTS

Late on 14 March, administrative and medical authorities met to formulate an emergency programme. Emphasis was laid on the importance of detecting and isolating all persons with recent gastro-intestinal upset, unexplained pyrexia or other unexplained illness.

It was realized that extra hospital accommodation would be needed and the local school was commandeered on 15 March to provide this.

Between 13 and 15 March, eighteen suspected cases were removed to various hospitals in the Canton of Valais.

In spite of the unexpectedness of the event, administrative and medical action was rapid and the Chief Medical Officer of the Canton was able to arrange for the Emergency Hospital to receive patients by Saturday 16 March.

No public announcement of the existence of the epidemic was made at first, but on 16 March the Canton considered it advisible to issue an official bulletin on the subject. Thus, the news became known throughout Switzerland and acted as warning to anyone who intended to come to Zermatt. Nevertheless it was difficult to stop the tourist traffic from abroad. Although it was not considered necessary to close the resort immediately, the situation was publicized through press, radio and television. On the same day precise instructions were given to the hotel managers about immediate measures to be taken and, in particular, newly arriving tourists were warned of the existence of a typhoid outbreak in the town and were given the option of cancelling their reservations.

By Sunday 17 March, no further removal to hospitals outside Zermatt was needed, but admissions to the Emergency Hospital suggested that the epidemic was probably distributed throughout the village.

The Director of the Institute for Microbiology of the University of Geneva visited Zermatt on Tuesday 19 March and drew up a plan for the operation of emergency hospital, laboratory, local hygiene and epidemiological services. This was submitted to the Chief of the Swiss Army Medical Corps. As a result a Sanitary Troop Detachment was established in Zermatt between 21 and 23 March. Arrangements were made for the removal of patients by rail, once the diagnosis of typhoid fever was confirmed.

Chlorination of the water supply was raised to 0·3 p.p.m. on 19 March and to 0·6 p.p.m. on 23 March.

It must be stated that, in spite of the warning about typhoid fever, tourist activity continued unabated up to 17 March. The Gornergrat Derby, Zermatt's most important winter event, was about to start when the news of the epidemic was released. It was felt that, as the participants in this event were already in Zermatt, it was unlikely that the magnitude of the outbreak would be materially influenced by allowing it to take place. As events showed, this decision was justified. As Fig. 1 shows, the week-end of 16 and 17 March, during which this Derby was held, was not associated with an especially large influx of tourists, so that the extra number at risk was not disproportionately large. Moreover, 88 % of the total number of cases were already ill at the time of the Derby, and sub-

sequent investigations suggested that the period of infection had terminated a few days before the end of February.

Information about the outbreak was officially released by the Federal Health Authorities on 18 March, but tourists continued to arrive and the sports activities of the village were maintained. Nevertheless, the impact of the news was such that an exodus of tourists rapidly got under way, so that by 25 March the tourist population had dwindled by several thousand people. Because of this exodus, and because of the need for disinfection of premises and of bacterial examination of employees and residual guests, and following the advice of the military authorities, the hoteliers decided to close all hotels on 30 March. Similar action was taken 2 days later by the proprietors of pensions and chalets. In numerical terms, during the week 17–24 March, departures exceeded arrivals by 4000, whereas in the preceding week departures were balanced by arrivals (see Fig. 1). In the week ending 30 March the net tourist population had shrunk by a further 2500 people.

The effect of the epidemic on tourist movements is shown in Fig. 1, where the net number is maintained at about 9000 up to 17 March, and falls steeply thereafter.

EPIDEMIOLOGICAL INVESTIGATIONS

Zermatt is traversed by a small river, the Vispa, which empties into the Rhone. At the beginning of the investigations a spot map of cases and suspects showed that the epidemic was widely distributed throughout the village, but later a concentration on the left bank of the Vispa was evident.

On 1 April, the physicians in ten Swiss hospitals were telephoned. They were asked to obtain precise details of the clinical history of the patients in their care, paying particular attention to the dates of onset of illness. By 3 April we had details of 112 cases. It is doubtful whether such information would have been forthcoming had the inquiries been delayed beyond this point. The inquiry revealed that the most probable infective period was from 14 to 18 February, with possible extension on either side of these dates.

Valuable epidemiological information was also obtained by interrogation of patients in the Emergency Hospital in Zermatt. The special advantage of these local investigations was that they could be combined with epidemiological inquiry in the village itself.

By 10 April a total of 162 cases had been notified, most of them Swiss citizens. Information from abroad rarely reached our local centre, but it was estimated that there were probably a further 100 to 200 cases of which we had hitherto no knowledge. The Canton Public Health Authority decided that, for an intimate study of the outbreak, maximum information should be obtained about the cases in other countries. Accordingly, contact was established with the Health Services of the United States, Germany, France and Britain. Official lists of patients were obtained from various countries and help for our investigation was freely offered. Dr R. T. Ravenholt, at that time Epidemiological Consultant to the U.S. Embassy in Paris, was extremely helpful in initiating contact with several countries. Visits to the British Ministry of Health and to the Enteric Reference Laboratory,

Colindale, London, arranged by the Swiss Federal Health Authority, were particularly valuable.

In addition, through the mediation of the Swiss National Tourist Office, details of some 200 cases were obtained.

These inquiries resulted in the identification of 437 cases of typhoid fever, infected in Zermatt. About 260 of these had left Zermatt before falling ill or at an early stage in the disease. Of these, 77 were Swiss subjects residing elsewhere in Switzerland; and 183 were foreigners. Our international liaison was so successful that we were able to obtain quite full personal and medical information about these cases. In particular, the hotels at which they stayed were identified and the duration of their visit was determined.

A precise calculation of the number of persons at risk was very difficult because we were dealing with a heterogeneous population: a stable component of inhabitants and employees on the one hand; and a floating population of tourists with a mean residence of 9·1 days on the other. The most reliable figures of tourist movement were obtained from the railway company. Figure 1 summarizes the tourist arrivals and departures from mid-January to 23 April 1963. As will be shown later, the probable period of infection lay between 16 and 24 February. This is indicated in Fig. 1.

A house-to-house investigation carried out by the writer produced the following result: (1) verification of the place of residence of all reported cases; (2) population at risk per house during the period of infection; (3) incidental information such as remarks about offensive smell and turbidity of the water during the critical period in the middle of February. The local tourist office provided the information needed about hotels.

In this way it was possible to define a population at risk for each residence.

EPIDEMIOLOGICAL FEATURES

Dates of onset

The number of cases with a clear-cut date of onset of illness in Zermatt or after returning home is indicated in Table 2, the most convenient analysis seeming to be on a basis of intervals of 4 days. The total figure of 405 includes 93 % of all notified cases (437) and the dates of onset of the cases fell between 21 February and 28 March. The last case in the entire series fell ill on 2 April.

Fig. 2A shows the distribution of these cases according to the precise dates of onset. This distribution is what would be expected from a single source of infection over a limited period. There were few cases at the end of February, an explosive increase at the beginning of March and a gradual decline thereafter. The succession of peaks of onset during the decline period may be due to the random distribution of incubation periods (which is expected) or to intermittency in the primary source. As is usual in typhoid outbreaks, there was no indication that secondary cases contributed materially to the total number.

Fig. 2B gives the cumulative percentage distribution of dates of onset for tourists (T) and inhabitants (I). Although this figure shows no apparent difference

Table 2. *Cases of typhoid fever with clear-cut onset date* (21 *February to* 2 *April* 1963 *grouped in* 4*-day intervals*)

Period	No.			%	No.			%		
	Guests	Inhabitants	Total	Total	Guests	Inhabitants	Total	Total	Cumulation	Span
21–24 Feb.	7	1	8	2·0					2·0	21 Feb.
25–28 Feb.	21	6	27	6·7	28	7	35	8·6	8·6	28 Feb.
1–4 Mar.	71	47	118	29·1					37·8	1 Mar.
5–8 Mar.	64	36	100	24·7					62·5	to
9–12 Mar.	46	28	74	18·3	181	111	292	72·1	80·7	12 Mar.
13–16 Mar.	14	18	32	7·9					88·6	
17–20 Mar.	12	10	22	5·4					94·1	13 Mar.
21–24 Mar.	9	7	16	4·0					98·0	to
25 Mar.–2 Apr.	3	5	8	2·0	38	40	78	19·3	100·0	2 Apr.
Total	247	158	405	100·1	247	158	405	100·0	—	—

in the rate of development of the outbreak between the two groups, a study of the three main subdivisions by date exhibited in the right-hand half of Table 2 suggests that the rate of development of the outbreak was more rapid in tourists than in inhabitants. This is supported by Fig. 2C, which gives the cumulative percentage distribution of onset dates in tourists and inhabitants, each group being regarded as complete within itself. The curve of tourist onset precedes that

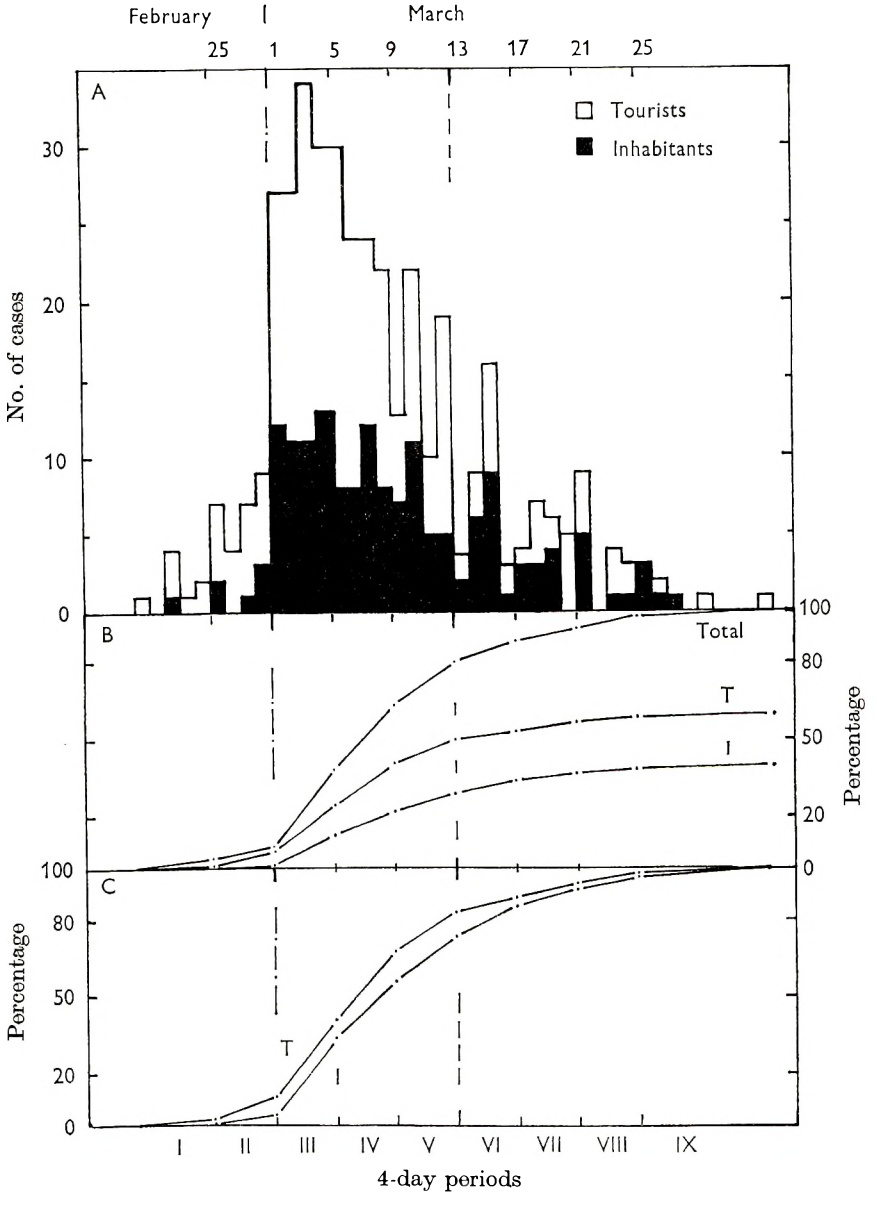


Fig. 2. Graphic analysis of onset dates in tourists and inhabitants. A, Distribution of onset in 405 patients presenting a clear-cut date of onset. B, Cumulative percentage distribution of tourists (T) and inhabitants (I) according to onset date. C, Cumulative percentage distribution of tourists and inhabitants according to date of onset, each group being regarded as complete in itself.

of inhabitants' onset by an interval of 2 days. In fact, by the end of February, 7·1 % of all tourist cases were already ill, whereas symptoms had begun in only 1·7 % of infected inhabitants. This difference is statistically highly significant ($P < 10^{-8}$). There is nevertheless no explanation to give for it up to the present.

Sex and age distribution

Unfortunately, the age and sex distribution of the total population at risk could not be determined. However, Fig. 3 presents a subdivision of 425 cases of the Zermatt outbreak according to sex and age. The overall distribution between

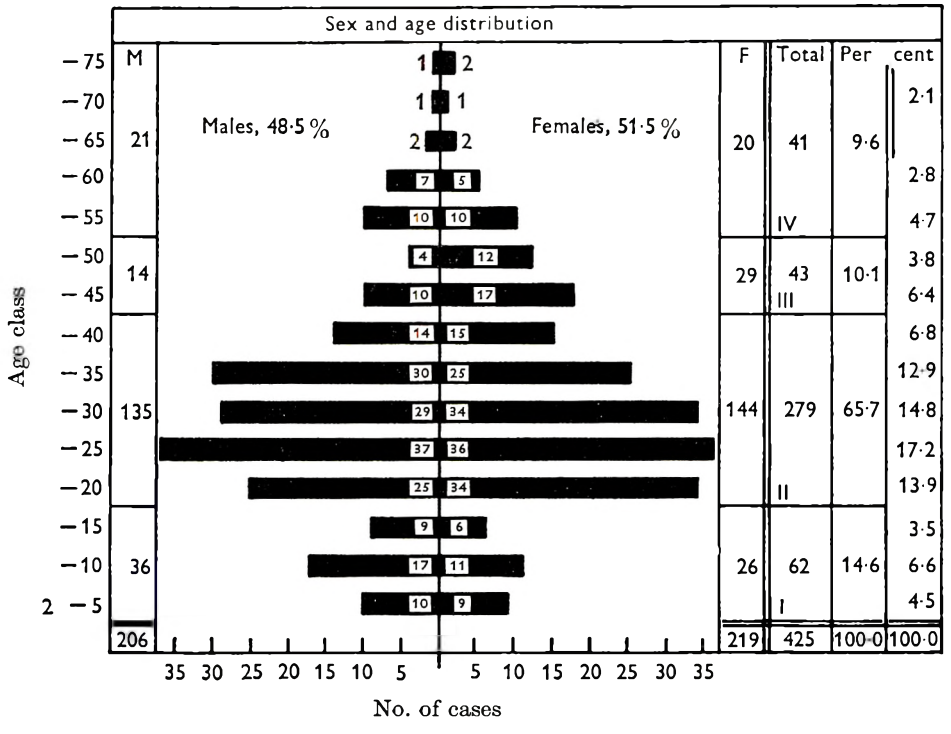


Fig. 3. Sex and age distribution of patients infected in Zermatt.

males and females was practically equal, but in the age group 41–50 there was a preponderance of infections in the females. This difference is on the margin of significance ($\chi^2 = 6{\cdot}87$, $n = 3$, $0{\cdot}1 > P > 0{\cdot}05$). In a winter sports centre the largest age group of visitors and employees lies between 16 and 45. It is therefore not surprising to find that 72 % of cases occurred within this age bracket. The age group 16–35 years included 58·8 % of cases with an equal distribution between males and females.

Incubation period and period of infection

The shape of the onset curve, as we stated before, suggests a widespread infection starting on a particular day, i.e. a definite interval before the explosive rise of cases. The earliest day of infection was calculated by determining the

earliest date on which a visitor infected with typhoid left Zermatt. This was 16 February. The end of the period of infection was indicated by the latest date on which a newly arrived visitor was infected. If we exclude two cases infected on 2 and 3 March, the end of the main period of infection was on 24 February.

Figure 4 gives the attack rate per thousand tourists analysed separately in terms of arrival and departure. The periodic fluctuations in the curves correspond to the week-end periods of greatest tourist activity.

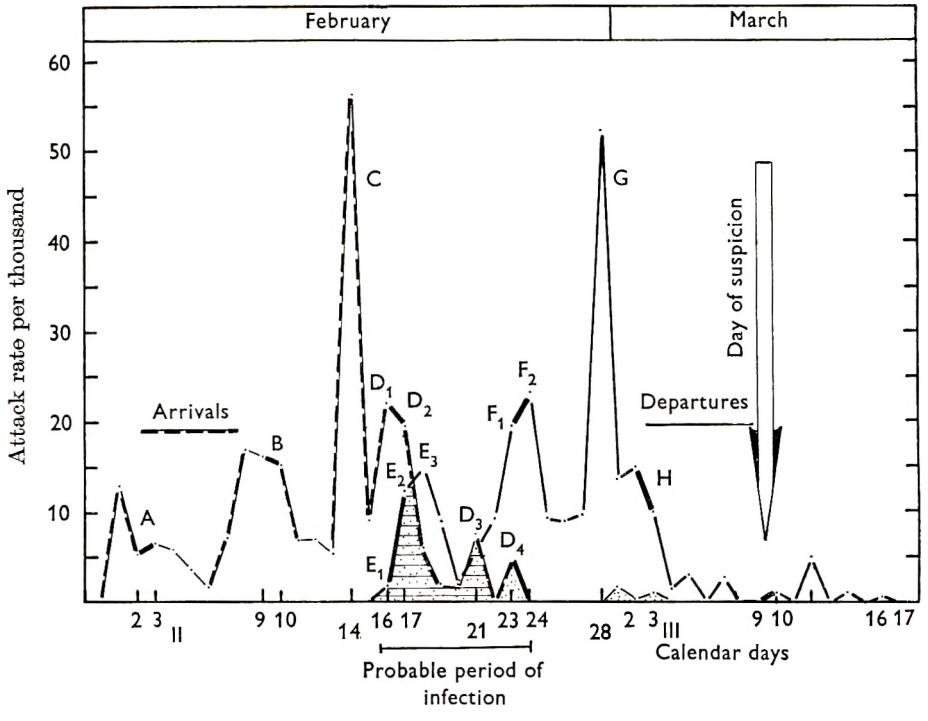


Fig. 4. Incidence of typhoid fever among guests according to arrival at and departure from Zermatt.

The region of overlap of the two curves indicates the minimum infective period. As has been mentioned, the first departing visitor to be infected left Zermatt on Saturday 16 February. Nineteen persons who left on 17 February and a further nine who left on 18 February developed typhoid fever. These figures correspond to a daily attack rate of 2, 13 and 14·9 per thousand departures respectively. Visitors leaving on the following week-end showed a much higher attack rate (23 and 24 March with 20 and 23 per thousand departures) which would be expected because they had been in Zermatt for a longer fraction of the total period of infection and therefore had a higher probability of being infected.

Turning now to the curve of arrivals, it is clear that the main infection period finished about 24 February, although two cases were infected who arrived on 2 and 3 March.

A question arises in relation to the distribution of initial infections in this outbreak. Are we dealing with a distribution of cases of which a regular number were

infected daily, or were most patients infected at the beginning of the infective period?

Sartwell (1950) found that an almost linear plot was obtained for a simultaneous and unique infection of all cases if the relative frequencies of incubation periods cumulated on a probit scale were plotted against the logarithm of the time of incubation.

Knowing with reasonable precision the infection date of the fraction who left at the start of the period of infection (Fig. 4, fraction E), it is convenient to consider their onset curve simultaneously as the distribution of maximum incubation

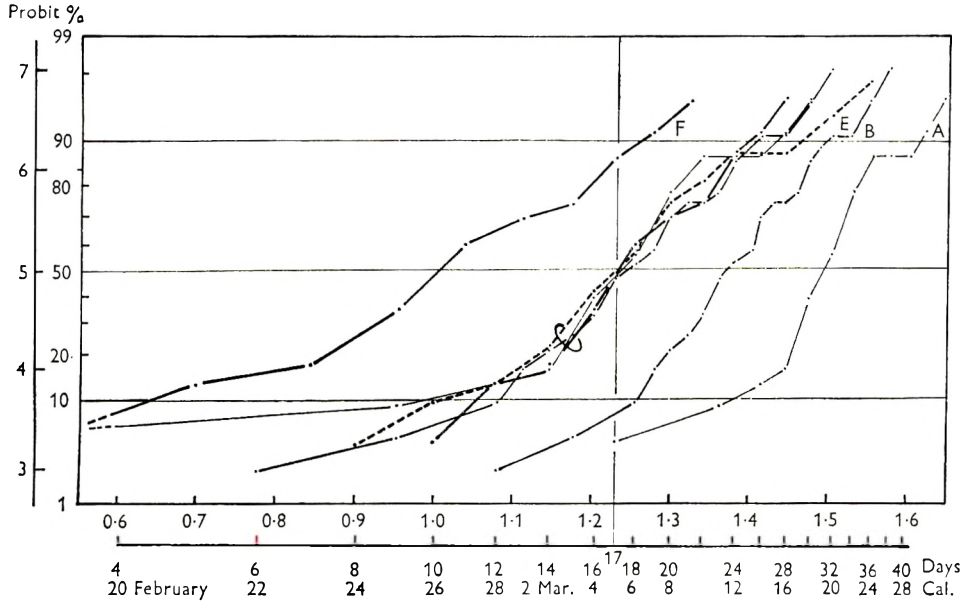


Fig. 5. Probit analysis of probable incubation periods. Cumulation of relative frequencies of incubation period groups on a probit scale; time of incubation in logarithms. *Curve E* (= best known infection date) represents the first leaving fraction with typhoid victims whose onset curve is considered as the most accurate distribution of incubation periods. The curve indicates a one-point epidemic with a median incubation period of 17 days and 11–25 days 80 percentile range. *Curves F, B, A* (= dates of infection unknown); see also Table 3. These curves are based on hypothetical infection dates and suggest one-point infections. Demonstration of a common and short infection period of all fractions: by shifting the individual curves by the difference from the best known mean incubation period (E = 17 days) all fractions fall together forming the bundle with its median incubation period of 17 days.

periods. In doing so a linear plot and a reliable median infective period of the outbreak should be obtained. Figure 5 gives the result of this procedure, the ordinate representing cumulative percentages of cases on a probit scale. The abscissa is the time scale on a logarithmic basis and simultaneously, for easier recognition, the arithmetical number of days of onset after the calculated initial infection (16 February). Curve E (Fig. 5), which is derived from fraction E (Fig. 4), shows that the mean incubation period of this group was 17 days with an 80 percentile range of

11–25 days. This rather long incubation period is what would be expected in a waterborne outbreak of typhoid fever where the average dosage with *S. typhi* is low.

The question arises as to whether the patients represented in the different peaks, corresponding to successive week-ends, were infected at the same time as those who left Zermatt on the week-end of 16 February. We postulated for practical purposes that all groups for which a definite date of infection was not known were infected either on the day of arrival or the day of departure. Tracing the frequency distribution of hypothetical incubation periods of these patients on normal probability paper, the curves should be straight lines with individual hypothetical median values. If most patients were infected at the same time, the difference of position of these curves from that of curve E, in relation to the abscissa, should correspond to the number of days by which every group's date of infection was under- or overestimated. Insertion of the corresponding corrected curves, carried out by moving them along the abscissa by a distance corresponding to the calculated differences, should then bring them into the vicinity of curve E, if the various fractions were infected at closely similar dates. In Fig. 5 the curves of earliest arrivals and of departures (corresponding to fractions A and F of Fig. 4) and curve B (fraction B of Fig. 4) are plotted against curve E, which is that of those known to have been infected between 16 and 18 February.

As an example we may consider the first arrivals in Zermatt who were infected. These are fraction A (Fig. 4) and the curve A (Fig. 5). We see in Table 3 that this fraction has a 31-day median incubation period if common infection is postulated on the day of arrival. The corresponding 80 percentile range is 24–41 days. The difference from the best known median incubation period (17 days) is 14 days, which is the span by which the curve A must be shifted to the left on Fig. 5 if the estimate of a median incubation period of 17 days is correct. This will give a mean incubation period of 17 days for fraction A with an 80 percentile range of 10–25 days and a date of infection on or about 16 February.

The same procedure applied to all fractions of Fig. 4 suggested, as is seen in Table 3, a median incubation period of 15–17 days and an 80 percentile range from 11–24 days.

This supports the hypothesis that, in spite of the fact that we know the minimum infective period to lie between 16 and 24 February, the majority of patients were infected during a more limited number of days early in the infective period.

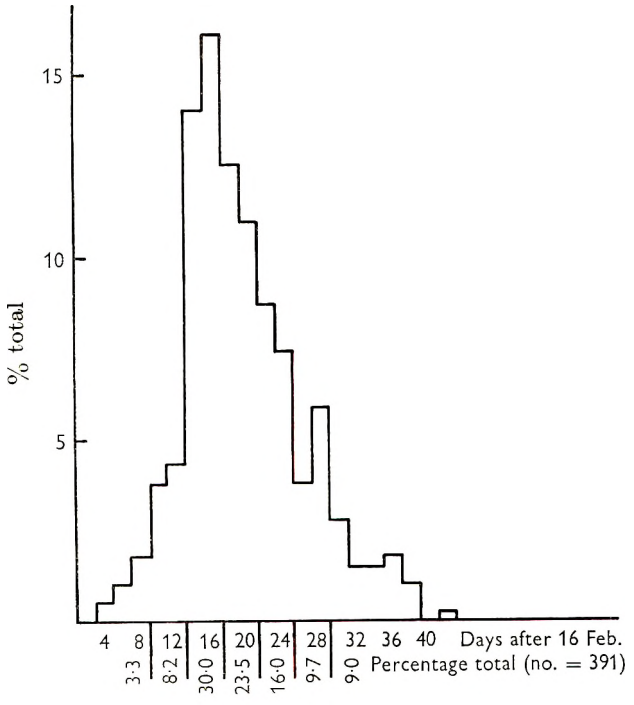
The fact that nine tourists who arrived on 23 February contracted typhoid fever indicates that infection was active on that date. With one exception these tourists stayed in the same hotel. No other tourist arriving 23 February was infected. It thus seems that the area of infection had become limited by that date (see Fig. 1).

As the infective period seems to have an initial concentration, the date of onset of each case can be regarded as a reasonably reliable guide to its incubation period, provided that the patient was in Zermatt on the week-end of 16 February. Obviously, those few who arrived in Zermatt later than that week-end must have their maximum incubation period limited by their dates of arrival. Taking into

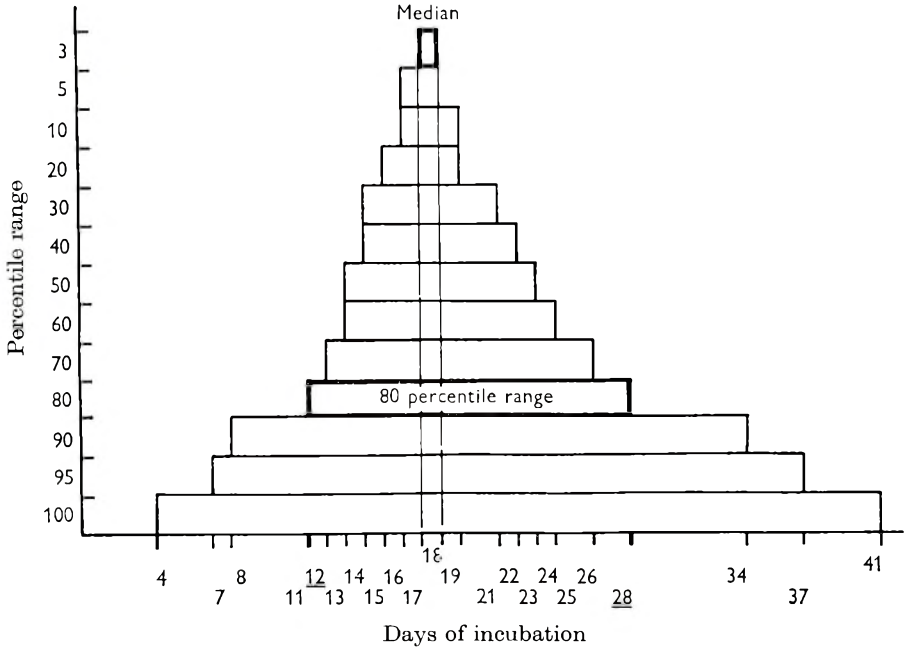
Table 3. *Attack rates and probable incubation periods in tourists in the Zermatt outbreak*

(Attack rates by arrival and departure day. Hypothetical and real (calculated) incubation periods for homogeneous fractions of tourist victims with regard to arrival and departure dates. Localization of beginning of infective period (16 February) and assumption of infection of most victims on 16, 17 and 18 February.)

	Date of		No.				Incubation period: Hypothetical			Incubation period: Calculated	
Fraction	Arrival	Departure	Arrivals	Departures	Cases	Rate per 1000	80 percentile range	Median	Diff.	80 percentile range	Median
A	1 Feb.	—	778	—	10	12·8	24–41	31	−14	10–25	17
	2 Feb.	—	1288	—	7	5·4					
	3 Feb.	—	1073	—	7	6·5					
B	9 Feb.	—	1548	—	25	16·2	18–30	24	− 7	11–23	17
	10 Feb.	—	1248	—	19	15·2					
C–1	14 Feb.	—	794	—	17	56·7	9–31	18	− 2	7–29	16
C–2					28		12–29	17	− 2	10–27	15
D–1	16 Feb.	—	1909	—	42	22·0	10–27	17	0	10–27	17
E–1	—	16 Feb.	—	1510	3	2·0					
D–2	17 Feb.	—	1470	—	29	19·7	11–24	16	0	11–24	16
E–2	—	17 Feb.	—	1456	19	13·0	11–25	17	0	11–25	17
E–3	—	18 Feb.	—	606	9	14·9					
F–1	—	23 Feb.	—	1165	23	19·7	—	—	—	—	—
F–2	—	24 Feb.	—	1719	40	23·3	2–15	10	+ 7	10–23	16
G–1	—	28 Feb.	—	952	22	52·5	−2–17	5	+12	10–29	17
G–2	—				28		−3–15	3	+12	10–27	15
H	—	2 Mar.	—	1611	25	15·5	−2–11	3	+14	12–25	17
		3 Mar.	—	1793	18	10·0					
							Determined incubation period of Zermatt incidence in tourists			11–24	16–17



A



B

Fig. 6. Frequency distribution of incubation periods. A, Percentage frequency distribution of cases according to calculated incubation periods. B, Percentile ranges of incubation periods.

account these factors, Fig. 6 was constructed to demonstrate the frequency distribution of incubation periods (Fig. 6A) and the corresponding percentile ranges (Fig. 6B). It must be emphasized that the right-hand portion of Fig. 6A is not so accurate as that to the left, because patients who were already in Zermatt on the week-end of 16 February could nevertheless have been infected on any day up to 24 February. The estimated incubation periods for these cases must thus be regarded as maxima. However, this does not materially affect the general distribution of real incubation periods. Fig. 6A shows that 70% of all cases have an incubation period between 13 and 24 days and only 11·5% have one of less than 13 days. Nearly 20% of all cases presented an incubation period exceeding 24 days, the median was found to be 18 days and the mode was 15 days (37 cases, 9·5% of the total).

Fig. 6B presents an analysis of the frequency distribution of incubation periods on a percentile basis. The maximum range is 4–41 days and the 90 percentile range 8–34 days. The median was 18 days and the 80 percentile range 12–28 days; these were probably the most accurate parameters of the distribution and are marked with a bold line in Fig. 6B. A long median incubation period and an 80 percentile range also on the long side are the characteristic features of this outbreak, which, as has been pointed out before, being waterborne, was almost certainly associated with low dosage of the infective organism. It is also interesting that the foregoing analysis of the entire outbreak could be applied with very small differences to the group of 31 cases represented in group E_{1-3} on Fig. 4.

GEOGRAPHICAL DISTRIBUTION

Distribution of nationalities

Tables 1 and 4 show that roughly three-fifths of all victims were Swiss; Great Britain had the highest figure for foreigners with 17·8%. Two-fifths of all cases

Table 4. *Distribution of cases by nationality*

	Nations		Cases	Per cent total
Residents	Switzerland	Zermatt	177	40·5
		Rest of Switzerland	77	17·6
Tourists	Other nations	Great Britain	78	17·8
		France	37	8·5
		Germany	33	7·6
		United States	14	3
		Remaining nations	21	5
		Total	437	100·0

were local residents. As Table 1 shows, the attack rate per thousand is three times as high in British tourists as in the remaining visiting nationalities (182/83,449 = 2·18 per 1000 guest-nights). The reason for this preponderance of British cases is not apparent, but it may be due to the fact that there was a high attack rate in the hotel in which the majority of British tourists were staying during the infection period.

Local distribution of cases

A provisional spot map drawn early in the outbreak showed a preponderance of cases on the left bank of the Vispa, with concentration in the lowest part of the village near the railway station. Fig. 7 shows the final distribution of cases. It is seen that 17·8 % were accommodated on the right bank with an evident concentration in two limited areas, whereas 82 % were staying on the left bank. There

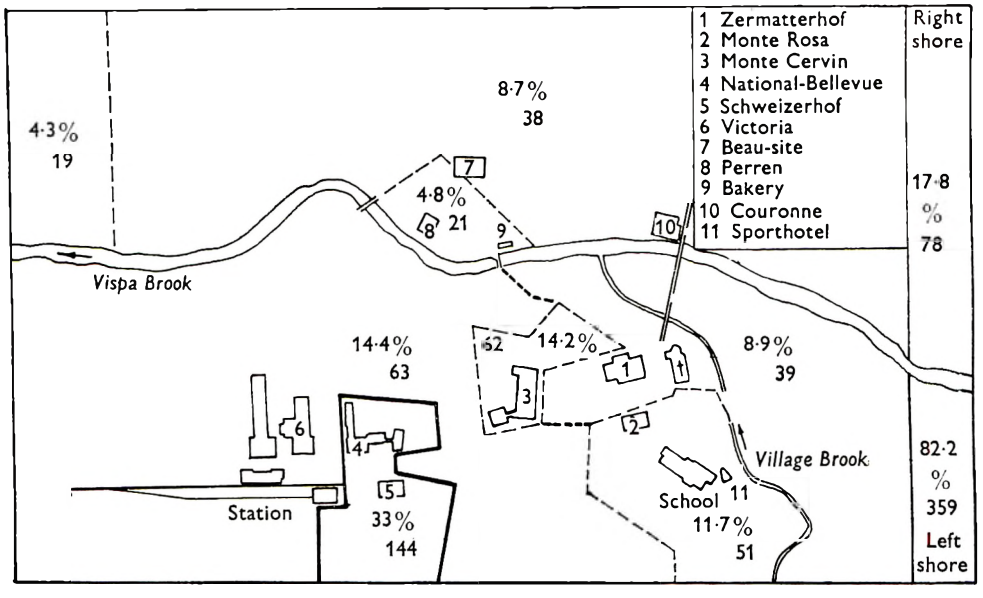


Fig. 7. Local distribution of cases (percentage total and number).

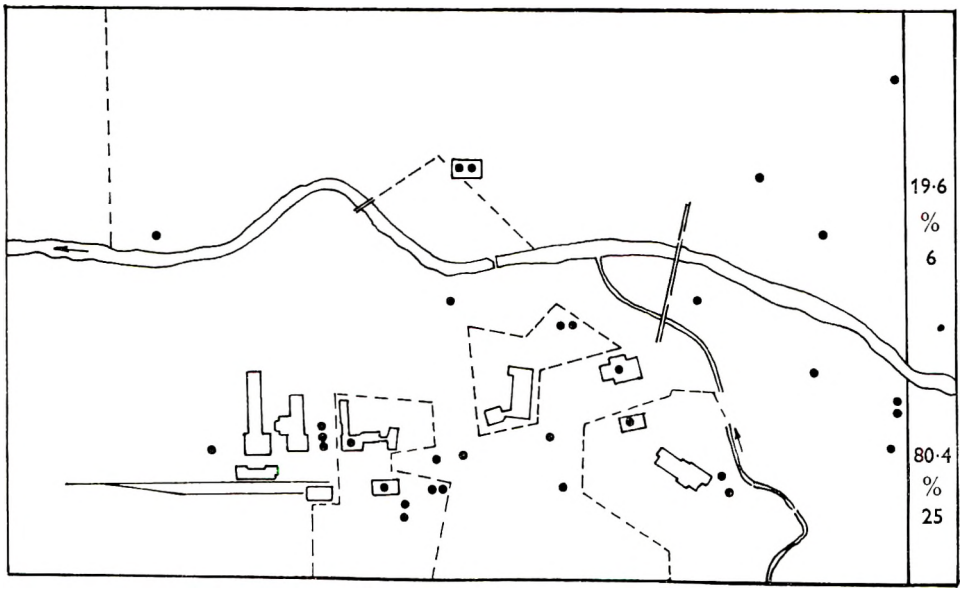


Fig. 8. Residence of tourist cases leaving on 16, 17 and 18 February. (Fraction E_{1-3}; first departures.)

were also obvious concentrations of cases on this bank; in particular, a limited area contributed 33 % (= 144 cases) of all cases. The left bank represents the original village and contains most of the hotels.

A study of the geographical distribution of cases known to have been infected at the beginning of the outbreak showed that infection was distributed throughout the village from the start with a concentration on the left bank (Fig. 8). This is indicative of a common vehicle.

THE WATER SUPPLY OF ZERMATT

As may be seen in Fig. 9, various catchment areas yield the community water. A series of nearly twenty lateral moraine springs flow in a more or less protected conduit to a collecting chamber from which two pipes arise, one supplying the

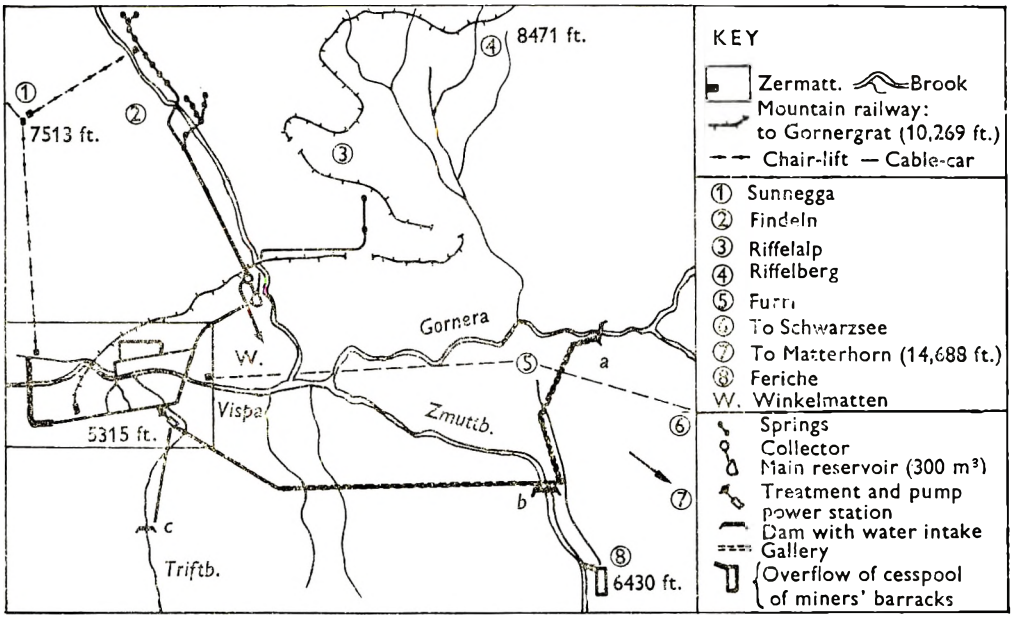


Fig. 9. The water sources of Zermatt.

nearby hamlet of Winkelmatten, the other conducting the excess to the main reservoir of Zermatt with a capacity of 300 m.³. The water of two other springs, collected on Riffelalp, is conducted directly to this reservoir. All this water is originally of good quality, but being surface water it is liable to repeated contamination. It must be emphasized that this raw water runs untreated into the general supply. Nevertheless, as Winkelmatten had no cases and as the right bank of the Vispa, which is essentially supplied by this water source, had the lowest attack rates, the Findeln catchment area may be excluded as a source of infection.

The second source of water is the catchment area of two glacier streams, the Gornera and Zmutt. From their respective dams the water is conducted in a joint main and serves first to provide power for the hydroelectric station of Zermatt, after which it is piped to the nearby water purification station.

The power station has another supply of water, the Triftbach; in the winter the

region from which this water comes is uninhabited, so that at the time of infection it was unlikely to be contaminated. This is not true for the Zmuttbach area. About 400 yards (365 m.) above the water intake there was at the time of the outbreak a hostel accommodating over 100 Italian miners employed on a hydroelectric project. In addition to the fact that these workers very often defaecated directly into the Zmuttbach and on its banks, the overflow of the cesspool into which the lavatory accommodation of the hostel drained ran directly into the Zmuttbach. This cesspool was found to be filled to overflowing during the critical period.

The water leaving the Zermatt hydroelectric station was purified by filtration through quartz sand followed by chlorination with a Hottinger injector apparatus into the storage chamber, which was beneath the station and had a capacity of 30 m.^{3}. Three sand filters were used, one of which had been installed as recently as 1962. The water engineer discovered two important faults in the design of the treatment station. On the one hand it was determined with precision that about 70 m.^{3} of unchlorinated water entered the general supply whenever the newly installed filter was cleaned (this was carried out every 1–2 days by reversed flow under pressure). The two older filters did not suffer from this fault. On the other hand, the holding time of the chlorinated water was found to be extremely short. It was calculated to be reduced to under 15 min. during the peaks of consumption (at about 6 p.m.).

Calculation of the total consumption of hypochlorite for the first 3 months in 1963 revealed that the chlorination on average was reaching only one-third of the required 0·2 p.p.m.

In addition, the result of one of the rarely executed water examinations was available. Samples were taken on 11 February after complaints had reached the Municipality concerning the high incidence of 'Zermatitis' (gastro-enteritis). The water was sampled before and after treatment. The pre-chlorination sample was heavily positive for *E. coli*, while that after chlorination was negative. It was discovered that the residual chlorine in the chlorinated sample was not neutralized before it was received for analysis. There was therefore a period of at least 10 hr. during which the chlorine could exert its sterilizing action. Thus, no reliance can be placed on the results of examination of the chlorinated sample. This examination was carried out on 11 February, that is, 5 days before the postulated start of the period of infection (16 February). The director of the Laboratory who carried out the examination added the comment that water of such a degree of initial contamination should not be used for drinking.

It may be added that the first decision on the initial day on which it was suspected that a typhoid epidemic was developing (see Fig. 1; 10 March) was to cut off the water from the Zmutt.

Distribution of cases according to the water supply

The water engineer subdivided Zermatt into *Sections* (designated A to U in Fig. 10A). Attack rates were calculated for each section and, with the water engineer's guidance, these sections were fused into *Regions* according to the origin of the water (Fig. 10B).

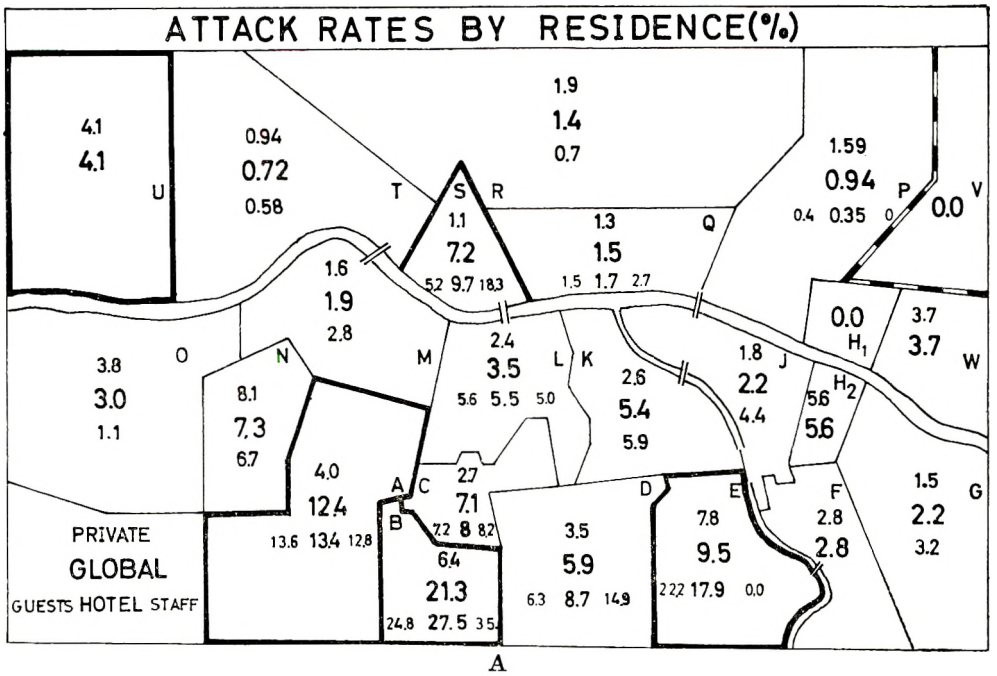


Fig. 10. A, Percentage attack rates by residence. B, Regional attack rates by water supply.

It can be seen at a glance that the region supplied by water from the treatment station showed a high attack rate, whereas that supplied by the Findeln source had a low attack rate. Thus in the region supplied essentially by water from the Zmutt and Gornera the morbidity rate (9·5 %) was about 9 times as high as that supplied essentially by Findeln water (1·1 %). The two systems are interconnected at three points and the origin of water at any point will therefore depend on the relative demand in the various areas.

To give a more accurate idea of the dynamics of the system the pipes are represented according to a volume coefficient scale; that is, their width is shown according to the square of their radii, and the corresponding coefficient, which is one-tenth of the figure, is given. For example, the coefficient '22' in relation to one of the three interconnexions ('*c*' in Fig. 10B) represents a value of 220 as the square of the radius of 14·83 cm. The diameter is therefore 29·66 cm.

On this basis the following observations may be made.

First, there is a quite uneven distribution of local static water in relation to various areas, the values ranging from 1 to 22. This means simply that more water was available from the larger mains. When it is remembered that internal resistance rises steeply with diminution in diameter, the preponderant supply of water from the larger mains is still further accentuated. This explains the relatively high attack rate in section U (I b′) on the right bank, which is predominantly supplied with water from the left bank through a wide-bored main. The water engineer calculated that this main held sufficient water to supply all of section U for 48 hr.; thus, it acted as a small local reservoir in which water could stagnate. This static water had to be consumed before a fresh supply could enter.

Secondly, as we have pointed out, the two banks of the Vispa had basically independent water supplies, the right bank from Findeln, the left from the Gornera–Zmutt–Trift streams. The contrasting attack rates on the two banks have already been mentioned (Region Ia: 1·1 % and Region IIIa: 9·5 %). Nevertheless, at certain times each bank may be supplied by water coming from the system of the opposite bank, as on the one hand water not immediately consumed on the left bank is pumped to the reservoir which supplies the right bank and, on the other, during the time of maximum demand on the left bank Findeln water runs into the left bank system. The supply of water to the right bank from the left bank system explains the occurrence of cases on the right bank. The points of low incidence on the left bank are those receiving the water predominantly from the right bank supply (Region II and IIIb with 2·6 and 3 % respectively).

Thirdly, no hydrodynamic explanation can be given for the high attack rate of the region situated in a bend of the Vispa River (Ib with 7·2 %). The water engineer stated that only one hotel, situated near the Vispa River, may be supplied essentially by left bank water and this during a limited time only. This hotel owns an adjacent bakery, employees of which take their meals with the hotel employees, and several sleep in the hotel. This hotel showed a much higher attack rate in its employees, including those of the bakery (32 %) than in its guests (7·2 %). We have as yet no explanation for this disparity (see Fig. 11).

The percentage attack rates subdivided according to water supply and popula-

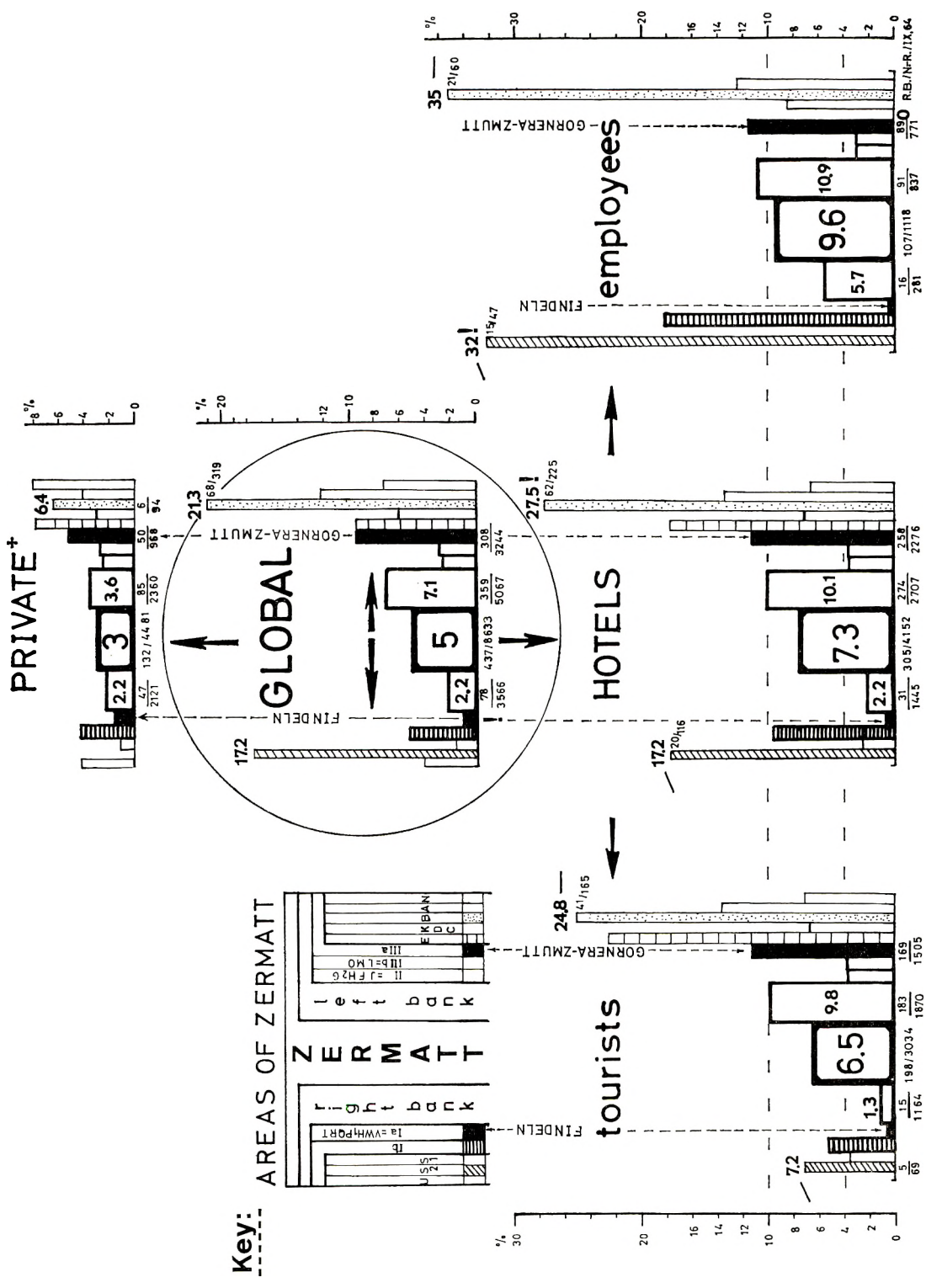


Fig. 11. Attack rates per cent by water supply and population category. The fractions present the number of cases (numerator) and the total number at risk (denominator).

tion category are represented in Fig. 11. This figure is a graphic summary of the entire outbreak. It should be studied from the centre circle in which the overall attack rate is shown to be 5 %. This figure shows that the left bank had an attack rate three times as high as that on the right bank (7·1 and 2·2 % respectively) and that the attack rate for hotels was more than twice as high as private houses (7·3 % and 3·0 % respectively) in which residents not employed in hotels and guests not booked in hotels were living.

INCUBATION PERIOD BY PLACE AND POPULATION FRACTION

Analysis of the incubation periods of guests and hotel employees yielded the surprising result that, whereas the median incubation period of the guests was found to be 16 days, that of the employees was 21 days. These hotels were situated in the areas A, B and C of Fig. 10A.

It was also found that the median incubation period increased as the source of water supply to their hotels received water from the right bank system. Thus, tourists in areas L, M and O had a median incubation period of 17 days, in F, G, H_2 and J 18 days and V, P, Q, R and T 20 days.

This may be due to reduced dosage with the typhoid bacillus as the water became progressively diluted with that from the right bank.

The finding of a 5 days' longer median incubation period for the employees than the tourists is consistent with the observation in Fig. 2C, which shows that the curve of onset of the tourists precedes that of the inhabitants. There is no evidence that there was any difference in dosage between tourists and employees. The only explanation that can be offered for the difference in incubation period, if it is significant, is that the hotel employees, many of whom came from regions of high typhoid incidence (southern Italy), may have had a basic immunity against typhoid fever, which retarded the development of the disease.

POSSIBLE ORIGINS OF INFECTION

We have demonstrated that this epidemic was due to water contamination. The question now arises of how the typhoid bacilli gained access to the water system. Two possibilities exist.

A labourer, aged 20 years, who had arrived from southern Italy on 6 February 1963, suddenly fell ill after working for 1 week on the hydro-electric project high above Zermatt. His symptoms were fever and gastro-enteritis. After a week in bed he was admitted to a Zermatt clinic and was transferred on 28 February to the hospital at Brig (see Fig. 1, case CH-206).

On 3 March a serological report stated that he had a TH titre of 1/400. It is known that from 20 February onwards he was treated with chloramphenicol. Examination of his excreta was not carried out until the end of February and this and all successive examinations were negative for the typhoid bacillus. The diagnosis of typhoid fever in this man hangs on the tenuous evidence of his single H-agglutination titration. Nevertheless, if he was indeed suffering from typhoid

fever whilst still living in the hostel, the typhoid bacilli he may have excreted could have contaminated the Zmuttbach.

The second hypothesis is based on two things. The writer's inquiries revealed that, for a few days before the beginning of the suspected period of infection, the tap water was 'cloudy, offensive smelling, oily and contained black particles'. Secondly, it was discovered in November 1963, that is about 6 months after the outbreak, while operations for enlarging the holding tank were in progress, that a nearby sewage pipe was broken and investigations with fluorescein established that sewage from this leak had been entering the holding tank. The complaints about the conditions of the water immediately preceding the infectious period, which were specifically located in the neighbourhood of the purification station, suggest that this leakage of sewage was already taking place at that time. If an incubating case of the disease, or a tourist who was a chronic typhoid carrier, or a local resident who was a chronic carrier, was excreting the typhoid bacillus into this sewage area at that time, the organism could easily have gained access to the water-holding tank.

In view of the fact that it had been suggested that the holding time was too short for effective chlorination, either of the possibilities mentioned could have resulted in contamination of the general water supply, with concentration on the left bank of the Vispa.

ANOMALIES

Apart from the Italian worker, three cases present us with a problem. These are shown at the top of Fig. 1.

(1) A 20-year-old girl who stayed in Zermatt from 1 to 21 January 1963 fell ill on 8 March (case CH-367). From 21 January to the time of onset of illness she was staying at a finishing school in Lausanne. If she was infected in Zermatt her incubation period was at least 42 days. While admitting this possibility it should be remembered that the usual extremes of the incubation period of typhoid fever lie between 5 and 28 days. This patient was infected with type E_1, which was the epidemic type, but as this is the commonest type on the European continent it offers no proof that she was infected in Zermatt.

(2) An employee of the hotel Riffelberg came to Zermatt for a staff carnival on 28 January (case CH-100). She was in Zermatt for that night only and returned to the hotel early on the following morning. She fell ill on 8 March and proved to be infected with phage-type E_1. If she was infected in Zermatt her incubation period was 36 days. This is again longer than average and raises the suspicion that she may have been infected in Riffelberg.

(3) A middle-aged woman (case CH-306) left Zermatt on 11 February (from section E on Fig. 10 A) to work as cook in Findeln (see Fig. 9). On 25 February she became ill and was finally diagnosed as suffering from typhoid fever, the organism again proving to belong to phage-type E_1. There can be little doubt that this patient was infected in Zermatt and because of this it can be concluded that the typhoid bacillus had already entered the water supply on 11 February. This patient complained that the tap water was offensive on the days before leaving

Zermatt. Combining the fact of her infection with the probability that there was sewage contamination of the water chlorination tank at that time, a suspicion strengthened by the complaints about the state of the water, it seems probable that the typhoid bacillus entered the water supply as early as 11 February, if not before. However, this early contamination may have been transient because the analysis of tourist cases indicated that the first day of continuous contamination was 16 February.

The two preceding cases can also be explained on the basis of a transient or minimal water contamination and, because under such circumstances their dosage with typhoid bacillus would probably be very small, their unduly long incubation periods would be explained.

DISCUSSION

The analysis establishes that the period of infection lasted for a limited span, 16–24 February, with a concentration during the first 3 days of this period. The epidemiological investigation shows clearly that the outbreak was waterborne and that the contamination of the water system took place at or before the treatment station. There seems to have been a possibility of some local intensification of attack rate because of unsatisfactory sanitary installation in some hotels, which allowed liquid to be aspirated from the sewage system into the water supply.

On examining the possibility of the origin of the outbreak, that is, the original source of the typhoid bacillus, two hypotheses present themselves:

(1) That the infection reached the Zmuttbach via the septic tank from the vicinity of the hostel housing predominantly Italian labourers. One of these was known to have a typhoid-like illness immediately preceding the onset of the outbreak. Unfortunately the investigation of this case was inadequate, and the organism was never isolated from him. The sole laboratory evidence that he may have suffered from typhoid fever was that a single examination of his serum revealed a TH titre of 1/400.

(2) The second hypothesis is that the treatment station or its reservoir became contaminated with *S. typhi*. At a rather late date it was discovered that there was a sewage leakage of long standing in the neighbourhood of the reservoir underlying the treatment station. It was established with fluorescein that sewage from this pipe leaked into this reservoir. Bearing in mind that several complaints about the unpleasant state of the drinking water immediately ante-dated the postulated period of infection, and that these complaints were located in the neighbourhood of the treatment station itself, it seems highly probable that the sewage leakage into the holding tank was occurring at that time. If an excretor of the typhoid bacillus was contributing to the sewage at that moment, the holding tank could have become contaminated with his organism.

It is impossible at this date to choose between these two hypotheses. What can be concluded however is that, had chlorination of the water been effective, the possibility of this outbreak occurring would have been greatly reduced.

SUMMARY

1. A description is given of the outbreak of typhoid fever in Zermatt in 1963.

2. There were 437 cases and three deaths, a case fatality rate of 0·7 %.

3. Information from 260 tourists established that the initiation of infection was explosive and allowed the period of infection to be determined.

4. The mean incubation period of various tourist fractions was probably between 16 and 18 days but was found to be significantly longer (20–21 days) for hotel employees.

5. The evidence favours a waterborne outbreak. Studies of the water supply showed that the catchment area and the surface streams and their water were liable to contamination.

6. One particular stream, the Zmuttbach, constituted a greater danger than the remainder.

7. The water purification at the treatment station was inadequate; in particular the holding time for chlorination was too short, and the required concentration of 0·2 p.p.m. was not reached consistently. In addition, there were periods during which completely unchlorinated water reached the general supply.

8. It was discovered, some months after the epidemic, that there was leakage of sewage, probably of long standing, into the chlorination tank. This seems to be the most likely source of the water contamination.

9. The typhoid excretor responsible for the outbreak was not discovered.

I am greatly indebted to Mr J. Bruderer, water engineer, for the valuable information he provided about the water supply, treatment station and sewage system. This information, which resulted from exhaustive studies, gives the most likely explanation of the cause of this outbreak. The encouragement of Dr P. Calpini, Chief Public Health Officer to the Canton of Valais was very helpful during the studies. I wish to thank Dr H. Reber, who commanded the Military Detachments concerned with the epidemic, for making his records freely accessible. The Ministries of Health of the countries mentioned in this report were all very co-operative in the collection of data. I am greatly indebted to Dr W. Kämpfen, Director of the Swiss National Tourist Office, whose collaboration simplified the collection of data. I gratefully acknowledge the information about the daily records so generously supplied by Mr A. Binz, Director of the railway company. The advice and encouragement of many specialists and scientists of the Swiss Universities were particularly helpful to me. The Swiss Embassy in France is acknowledged for its valuable technical assistance. The statistical calculations were carried out on a Tetractys calculator made available by the kindness of Messrs Olivetti in Paris. Finally, I wish to record my deep gratitude to Dr E. S. Anderson, Director of the Enteric Reference Laboratory, Colindale, London, for his help in the studies of this outbreak from the beginning, and for his guidance in the preparation of this report.

RÉSUMÉ

1. L'auteur décrit l'épidémie de fièvre typhoïde survenue à Zermatt en 1963.

2. 437 cas ont été enregistrés, dont trois mortels, ce qui représente un taux de mortalité de 0,7 pour cent.

3. Les informations recueillies sur les 260 touristes infectés ont permis de déterminer le caractère explosif de l'épidémie et d'en fixer la période d'infection.

4. La période d'incubation moyenne estimée parmi certains groupes de touristes se situait probablement entre 16 et 18 jours, alors que celle calculée parmi les employés d'hôtels a été nettement plus longue (20–21 jours).

5. Les faits recueillis parlent en faveur d'une épidémie hydrique à caractère explosif et de courte durée. Les investigations menées à chef sur les diverses sources d'eau ont démontré que la région de captage et les torrents pouvaient être infectés.

6. Le torrent de Zmutt constituait, en particulier, un danger de contamination plus grand.

7. La purification de l'eau dans la station d'épuration laissait à désirer. Le temps de contact de l'eau avec le chlore a été estimé trop court et la concentration minimale de 0·2 mg/l. n'a pas été atteinte régulièrement. De plus, durant certaines périodes, de l'eau non chlorée entrait dans le réseau d'eau potable.

8. Quelques mois après l'épidémie, une infiltration d'eaux usées a été découverte dans la chambre de chloration. Il s'agit certainement d'une infiltration ancienne qui semble être la source la plus probable de la contamination de l'eau.

9. L'excréteur de germes responsable de cette épidémie n'a pas été déterminé.

ZUSAMMENFASSUNG

1. Der Autor bringt eine Epidemiographie der Typhusepidemie des Frühjahres 1963.

2. Es wurden insgesamt 437 Erkrankungsfälle gemeldet, 3 davon verliefen tödlich, was einer Letalität von 0,7 vH entspricht.

3. Die Bearbeitung der Angaben von 260 erkrankten Touristen gestattet es, die Epidemie als Explosivepidemie zu identifizieren und die Expositionszeit festzulegen.

4. Die mittlere Inkubationszeit bei mehreren Touristengruppen lag wahrscheinlich zwischen 16 und 18 Tagen und war signifikant kürzer als die bei Hotelangestellten mit 20–21 Tagen.

5. Die epidemiologische Analyse deutete auf eine explosionsartige, kurzdauernde Trinkwasserepidemie hin.

6. Die Untersuchungen der Trinkwasserversorgungsanlagen deuteten darauf hin, daß das Quellgebiet und die Quellbäche möglicherweise einer Verunreinigung ausgesetzt worden waren. Dies gilt besonders für den Zmuttbach.

7. Die Wasseraufbereitung ließ zu wünschen übrig, insbesondere war die Chloreinwirkzeit zu kurz, und auch die minimale Chlorkonzentration von 0·2mg/l wurde nicht immer erreicht.

8. Einige Monate nach Erlöschen der Epidemie konnte festgestellt werden, daß Abwässer in den Chlorungstank eingedrungen waren. Hierin scheint auch der Grund für die Trinkwasserverschmutzung gelegen zu haben.

9. Ein bestimmter Bakterienausscheider, auf den die Epidemie zurückging, konnte nicht ermittelt werden.

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