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An *in vitro* comparison of the effect of some antibacterial, antifungal and antiprotozoal agents on various strains of *Mycoplasma* (pleuropneumonia-like organisms: P.P.L.O.)

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INTRODUCTION

Over the last 20 years many workers, mainly in the United States, Canada, Britain, Scandinavia and Australia, have sought suitable drugs to inhibit growth of organisms of the Pleuropneumonia group (P.P.L.O.) or Mycoplasmataceae. Most of the work has been carried out *in vitro* on the mycoplasmata of bovine, caprine, rodent or human origin, and *in vitro* or *in ovo* on those of avian origin. Summaries of the findings of this previous work are given in Tables 1 and 2.

The purpose of the present study was twofold: first, to discover whether representative collections of *Mycoplasma gallisepticum* strains from Britain and other countries were similar in their sensitivity to drugs; and, secondly, to find out whether *M. gallisepticum* drug sensitivities differed significantly from drug sensitivities of some other more typical members of the mycoplasmata; such differences might reflect differences in the structure of the various strains of *Mycoplasma* at present included in the single genus by Edward & Freundt (1956).

Our particular interest has lain in the apparent differences between the non-pathogenic avian mycoplasmata and the pathogenic coccobacilliform bodies of Nelson (1936*a–d*) which were named *Mycoplasma gallisepticum* by Edward & Kanarek (1960). Strain X 95 of Markham was taken as the type-species of this latter group, and so far all pathogenic strains tested in this laboratory have belonged to this distinct serological group (Chu & Newnham, 1959). Up to 1962 this was the only serotype known to agglutinate erythrocytes of avian and mammalian origin. But Yoder & Hofstad (1962) in the United States, and Roberts (1963) in Britain, have since described two new serotypes (distinct from each other and from *M. gallisepticum*) which may also agglutinate avian erythrocytes and which were isolated from air-sac lesions in chickens and turkeys.

M. gallisepticum strains differ from the more typical members of the mycoplasmata mainly in morphology, but Adler (1964) has listed a number of other differences. To gain more fundamental information on their supposed or actual differences, a study of this kind should be associated not only with studies in biochemistry and biophysics (Leach, 1962; Razin, 1963*c*; Razin, Argaman & Avigan, 1963; Morowitz *et al.* 1962), but also with serology, immunochemistry (Fowler, Coble, Kramer & Brown, 1963; Lemcke, 1964) and detailed cytology

Table 1. *Summary of earlier reports of inhibition of* Mycoplasma *by drugs* in vitro

Authors and date	No. of strains	Name and/or origin of strains	Type of medium used	Minimum inhibitory concentration	Minimum lethal concentration	Sensitivities expressed as μg. of drugs per ml. of medium															
						Streptomycin	Dihydrostreptomycin	Tetracycline	Chlortetracycline	Demethylchlortetracycline	Oxytetracycline	Chloramphenicol	Erythromycin	Spiramycin	Tylosin	Kanamycin	Sodium aurothiomalate	Nitrofurans	Nystatin	Polymixin
Paine *et al.* 1948 *a*	1	Human G.U.	Liquid	—	√	—	—	—	0·25	—	—	—	—	—	—	—	—	—	—	—
Hatch, 1949	6	Human G.U.	Liquid	—	√	25	—	—	25	—	—	—	—	—	—	—	—	—	—	No action
	1	Rat lung	Liquid	—	√	No action	—	—	25	—	—	—	—	—	—	—	—	—	—	No action
Leberman *et al.* 1950	7	Human G.U.	Liquid	√	—	0·1–15	15– > 200	—	25– > 200	—	—	15– > 100	—	—	—	—	—	—	—	—
Leberman *et al.* 1952	7	Human, G. U.	Liquid	√	—	—	—	—	—	—	0·1–0·5	—	—	—	—	—	—	—	—	—
Robinson *et al.* 1952	28	Human G.U.	Liquid	—	√	128–4096	—	—	8– > 256	—	4–256	16–512	—	—	—	—	16–128	—	—	—
			Solid	C.I.C.	—	2–128	—	—	2–16	—	0·25–2	4–16	—	—	—	—	16–128	—	—	—
		Rat arthritis (L 4) *M. arthritidis*	Liquid	—	√	2048	—	—	> 256	—	256	512	—	—	—	—	2048	—	—	—
			Solid	C.I.C.	—	128	—	—	16	—	1·0	8	—	—	—	—	2048	—	—	—
Melén 1952	20	Human G.U.	Liquid	√	—	5–10	20– > 80	—	0·32–1·25	—	0·16–0·63	2·5–10	—	—	—	—	—	—	—	—
Keller & Morton, 1953	3	Human G.U.	Liquid	√	—	—	—	—	—	—	—	—	> 200	—	—	—	—	—	—	—
Harkness & Bushby, 1954	6	Human G.U.	Solid	√	—	10–40	—	1·5–3·0	25–50	—	0·4–1·5	12–25	100–150	—	—	—	—	—	—	—
Blyth, 1958	47	Human G.U. *et al.*	Solid	√	—	4–32	—	0·25–0·5	0·5–4	—	0·5–1·0	4–8	> 64	2–16	—	—	—	—	—	—
Nasemann & Röckl, 1960	**	Human G.U.	Liquid	√	—	Good effect	—	—	0·5	—	—	Good effect	—	—	—	—	—	—	—	—
				—	√				5–10											
Robinson *et al.* 1956	**	Human G.U.	HeLa and conjunctival cell lines	—	? √	256	—	—	—	—	4	—	—	—	—	—	—	—	—	—
Kuzell *et al.* 1949	1	Rat arthritis (L 4) *M. arthritidis*	Liquid	√	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—
			—	—	√	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—
Hearn *et al.* 1959	**	T.C.C.	Solid (disks)	—	—	Resistant	—	—	Sensitive	—	Sensitive	Less sensitive	—	—	—	—	—	Less sensitive (N.F)	—	—
			Human and mammalian cell lines	—	√	—	—	—	100–200	—	—	—	—	—	—	—	—	—	—	—
Fogh & Hacker, 1960	**	T.C.C.	Amnion and HeLa cell lines	—	—	—	—	—	—	—	—	—	—	—	—	Inhib. by 200 for 2 weeks	—	—	—	—

Table 1 (*cont.*)

Authors and date	No. of strains	Name and/or origin of strains	Type of medium used	Minimum inhibitory concentration	Minimum lethal concentration	Sensitivities expressed as μg. of drugs per ml. of medium: Streptomycin	Dihydro-streptomycin	Tetracycline	Chlortetra-cycline	Demethylchlor-tetracycline	Oxytetracycline	Chloramphenicol	Erythromycin	Spiramycin	Tylosin	Kanamycin	Sodium aurothiomalate	Nitrofurans	Nystatin	Polymixin
Pollock *et al.* 1960	15	T.C.C.	Mammalian cell lines	—	—	—	—	—	—	—	—	—	—	—	—	Inhib. by 100 for 2 days to 3 weeks	—	—	—	—
Kenny & Pollock, 1963	4	T.C.C.	Human and mouse cell lines	—	—	—	—	—	—	—	—	—	—	—	—	Inhib. by 400 for 3 days	—	—	—	—
Pollock *et al.* 1963	66	T.C.C.	FE and HE cell lines	—	—	—	—	—	—	—	—	—	—	—	—	Inhib. by 100 for 5 hr.	—	—	—	—
Rouse *et al.* 1963	66	T.C.C.	Human cell lines	—	—	—	—	—	—	—	—	—	—	—	—	Inhib. by 50 for 2–3 weeks	—	—	—	—
Carski & Shepard, 1961	7	6 T.C.C., 1 G.U.	Solid (disks)	—	—	Resistant to 10	—	Sensitive to 30	Sensitive to 30	—	Sensitive to 30	Sensitive to 30	Resistant to 15	—	—	—	—	—	No action	No action
			HeLa cells	✓	—	—	—	0·5	—	—	—	8	—	—	—	—	—	—	—	—
Collier, 1957	66	T.C.C. (human G.U.)	HeLa cells	—	—	Resistant to 80	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Nelson, 1960	1	T.C.C.	HeLa cell lines	—	—	Resistant to 100	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Nelson, 1960	1	Mouse infectious catarrh *M. pulmonis*	HeLa cell lines	—	—	Sensitive to 100	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Kingston *et al.* 1961	66	Baton agent *M. pneumoniae*	Monkey kidney cells	✓	—	—	—	—	—	1·5	—	—	—	—	—	—	—	—	—	—
Lecos & Sperling, 1955	1	Pathogenic avian	Solid (disks)	—	—	—	—	'Good effect'	'Good effect'	—	—	'Less effect'	—	—	—	—	—	—	—	—
Yamamoto & Adler, 1956	10	Pathogenic avian	Liquid	✓	—	<6·25–12·5	3·13–>100	<1·56–6·25	1·5 100	—	<1·56–25	—	<1·56–100	—	—	—	—	Inactive (FZ and NF)	—	—
				—	✓	6·25–500	6·25–>500	1·56–>250	6·25–>250	—	<1·56–>250	—	1·56–>250	—	—	—	—	Inactive (FZ and NF)	—	—
Fahey, 1957	1	Turkey sinusitis	Liquid	—	✓	>100	—	—	25–>25	—	5–25	—	—	—	—	—	—	—	—	—
Domermuth & Johnson, 1955	1	Pathogenic avian (A 5967)	—	✓	—	0·01	—	—	0·1	—	0·01	0·1	—	—	—	—	—	0·1 (FZ) 10 (FZ)	—	—
			—	—	✓	10	—	—	100	—	10	100	—	—	—	—	—		—	—

Table 1 (*cont.*)

Authors and date	No. of strains	Name and/or origin of strains	Type of medium used	Minimum inhibitory concentration	Minimum lethal concentration	Sensitivities expressed as μg. of drugs per ml. of medium: Streptomycin	Dihydrostreptomycin	Tetracycline	Chlortetracycline	Demethylchlortetracycline	Oxytetracycline	Chloramphenicol	Erythromycin	Spiramycin	Tylosin	Kanamycin	Sodium aurothiomalate	Nitrofurans	Nystatin	Polymixin
Domermuth, 1958	1	Pathogenic avian (Winchester)	Liquid	√	—	0·1–1·0	--	0·1	—	—	—	0·1–1·0	—	—	—	—	—	0·1–1·0 (FZ)	—	—
				—	√	1000	—	> 10	—	—	—	10	—	—	—	—	—	10 (FZ)	—	—
Domermuth, 1960	1	Pathogenic avian (Winchester)	Liquid	√?	—	1000	—	10	—	—	—	100	1·0	—	—	—	—	—	—	—
				—	√?	> 10,000	—	100	—	—	—	100	10	—	—	—	—	—	—	—
Olesiuk & van Roekel, 1959	1	Pathogenic avian	Liquid	—	√	—	—	—	—	—	0·1	—	—	—	—	—	—	—	—	—
Osborn *et al.* 1960	1	Turkey sinusitis	Liquid	√	—	3·13	6·25	1·56	1·56	—	0·78	—	100	—	—	—	—	—	—	—
Gross, 1961	1	Pathogenic avian (Winchester)	Liquid	√	—	—	—	100–1000	—	—	—	—	0·01	—	—	—	—	10 (FZ) 1·0 (FT)	—	—
Cook *et al.* 1963	1	Turkey sinusitis	Liquid	√	—	—	—	—	—	—	—	—	—	0·5	—	—	—	—	—	—
Cook & Inglis, 1964	1	Pathogenic avian (A 514)	Liquid	√	—	—	—	—	—	—	—	—	0·06	0·25	—	—	—	—	—	—
Inglis (pers. comm.)	1	Pathogenic avian (A 514)	Liquid	√	—	—	—	—	—	—	—	—	0·06–1·0	0·125–4·0	0·008–0·125	—	—	—	—	—
Adler *et al.* 1956	2	Goat lung (K)	Liquid	√	--	100	> 12·5–100	1·15–50	> 25–50	—	1·56–> 50	—	< 1·56–6·25	—	—	—	—	—	—	—
		Goat arthritis (KS)	Liquid	--	√	100	50–100	50	50	—	25–> 50	—	1·56–50	—	—	—	—	—	—	—
	5	Sheep lung	Liquid	√	--	100	100	3·13–6·25	12·5–25	—	1·56–3·13	—	50	—	—	—	—	—	—	—
				—	√	100	100	6·25–25	50	—	3·13–6·25	—	50	—	—	—	—	—	—	—
Hamdy *et al.* 1957	2	Lamb pneumonia	Liquid	—	√	No action	—	—	No action	—	40–50	—	2500–3000	—	—	—	—	—	—	—
	2	Turkey sinusitis	Liquid	—	√	0·4–1·0	—	—	0·6–1·5	—	0·5	—	0·09	—	—	—	—	—	—	—
Turner, 1960	1	*M. mycoides* var. *mycoides* (V 5)	Liquid	√	—	7·8	—	0·25	15·6	—	7·8	—	3·9	—	—	—	—	12·5 (NF)	—	—
Pak (pers. comm.)	2	Goat pleuro-pneumonia	Liquid	—	√	—	—	5	> 100	—	10	—	—	—	< 0·5–1·0	—	—	—	—	—
Hudson (pers. comm.)	2	*M. mycoides* var. *mycoides*	Liquid	√	—	—	—	—	—	—	—	—	—	—	0·07	—	—	—	—	—
Razin, 1963 *b*	2 1 1 1 1	Saprophytic Human oral Bovine Goat *M. gallisepticum* A 5969	Liquid	√	—	—	—	—	—	—	—	—	—	—	—	—	—	—	> 125	—
Lampen *et al.* 1963	1	*M. gallisepticum*	Liquid	√	—	—	—	—	—	—	—	—	—	—	—	—	—	—	> 100	—

Key. T.C.C., tissue culture contaminant; G.U., genito-urinary tract; C.I.C., concentration causing complete inhibition; ∞, unknown number; ?, not clear; FZ, Furazolidone; NF, Nitrofurazone; FT, Furaltidone.

Table 2. *Summary of earlier reports on inhibition of* Mycoplasma *by drugs* in ovo

Authors and date of publication	Source and description and no. of strains	Drugs: Streptomycin or Dihydrostreptomycin	Chlortetracycline	Oxytetracycline	Chloramphenicol	Erythromycin	Kanamycin	Organic arsenicals	Sodium aurothiomalate	Polymixin
Wong & James, 1953	Pathogenic avian	Some action	Some action	Best action	No action	—	—	—	—	No action
Gross & Johnson, 1953	5, pathogenic avian	Prolonged life of embryos	Prolonged life of embryos	Prolonged life of embryos	Little action	—	—	—	—	—
Yamamoto & Adler, 1956	2, pathogenic avian (C and F)	Least action	Some action	Some action	—	Best action	—	No action	—	—
Hamdy *et al.* 1957	2, pathogenic avian	—	—	Some action	—	Best action	—	—	—	—
Adler *et al.* 1956	2, caprine (K: pneumonia; KS: arthritis)	—	Prolonged life of embryos	Prolonged life of embryos	—	Prolonged life of embryos	—	Prolonged life of embryos (KS only)	—	—
Switzer, 1953	*M. hyorhinis* (porcine)	50 mg./ml. protected 3/12 embryos	50 mg./ml. protected 10/12 embryos	5 mg./ml. protected 12/12 embryos	—	—	—	—	—	—
Naiemann & Röckl, 1960	Human genito-urinary	—	Inhibited by 0·5 μg./egg; killed by 5 μg./egg	—	—	—	—	—	—	—
Eaton, 1950	Mac and De strains of Eaton agent (*M. pneumoniae*)	—	Reduction of agent in yolk sacs	—	—	—	—	—	—	—
Eaton *et al.* 1951	Mac & De strains of Eaton agent (*M. pneumoniae*)	—	—	—	1 dose of 5 mg. 1 hr. after infn. inhibited multiplication	—	—	—	—	—
Eaton & Liu, 1957	Mac and FH strains of Eaton agent (*M. pneumoniae*)	MIC for Mac = 1000 μg. MIC for FH = 125 μg.	—	—	—	—	—	—	—	—
Marmion & Goodburn, 1961	Hetter (FH) strain of *M. pneumoniae*	—	—	—	—	—	—	—	1 dose of 25–30 mg. reduced no. of organisms	—
Goodburn & Marmion, 1962	Hetter (FH) strain and Bethesda PI 898 strain of *M. pneumoniae*	—	—	—	—	—	50 mg. inhibited specific antigen formation	—	1 dose of 25–30 mg. reduced no. of organisms	

combined with electron microscopy. A start has already been made in this laboratory using electron microscopy and agar-gel diffusion techniques and publication of our findings will follow in due course.

MATERIALS AND METHODS

Culture medium

The basal medium used throughout the work was Brucella broth and Brucella agar prepared by Albimi Laboratories Inc., Brooklyn, New York. The liquid medium was modified in the following way: 28 g. of the powder was dissolved in 100 ml. of distilled water and the solution was dialysed against 900 ml. distilled water. After 48 hr. the dialysate was made up to a final volume of 1 l. with distilled water and the pH adjusted to 7·0. To each 100 ml. of the medium was added 0·2 ml. penicillin containing 100,000 units per ml. and 1·0 ml. of a 1 in 80 solution of thallium acetate. Finally, Andrade's indicator was added together with 0·1% glucose and 15% unheated sterile horse serum. The complete medium was subsequently sterilized by passage through a Seitz-EK filter. The manufacturer's instructions for reconstituting the solid medium were followed exactly except for the addition of glucose, horse serum and the same concentration of antibiotics as used in the liquid medium.

Origin of strains of Mycoplasma, *etc.*

Twenty coccobacilliform strains of *M. gallisepticum* were tested together with sixteen strains of classified and unclassified mycoplasmata from various sources in Britain and other countries. L 1, the stable L-form of *Streptobacillus moniliformis*, obtained from the Lister Institute, was included for comparison. Summaries of details of the strains are presented in Tables 3 and 4.

Drugs and antibiotics tested

The antibacterial, antifungal and antiprotozoal agents used in this study are listed below:

Tylosin tartrate (Tylan)
Demethylchlortetracycline hydrochloride (Ledermycin)
Chlortetracycline hydrochloride (Aureomycin)
Tetracycline hydrochloride (Achromycin)
Oxytetracycline hydrochloride (Terramycin)
Spiramycin adipate (Rovomycin)
Erythromycin lactobionate (Erythrocin)
Chloramphenicol (Chloromycetin)
Streptomycin sulphate
Kanamycin sulphate (Kannasyn)
Ethidium bromide
Prothidium bromide
Antrycide methyl sulphate
Furazolidone

Table 3. *Strains of* Mycoplasma gallisepticum

Name of strain	Source	Origin	Date of origin	Remarks and references
A 197 A 101 A 202 A 303	Infectious turkey sinusitis	H. P. Chu (Cambridge)	1956 1956 1957 1958	Chu & Newnham (1959) A 301: Chu & Newnham (1959)
BMO A 733	Infectious turkey sinusitis	A. G. Newnham (Cambridge)	1961 1961	— Newnham, Ostler & Chu (to be published)
A 108 A 141 A 333	Sinus exudate (Nelson's Fowl Coryza)	H. P. Chu	1956 1957 1958	Chu & Newnham (1959)
A 514 A 752	Sinus exudate (Nelson's Fowl Coryza)	H. P. Chu & A. G. Newnham A. G. Newnham	1958 1961	From same farm as A 333: Newnham (1963) —
A 5969	Chicken with 'C.R.D.'	H. van Roekel (Massachusetts)	1951	Jungherr, Luginbuhl & Jacobs (1953)
X 95	Tracheal and air sac tissues of chickens with 'C.R.D.'	F. S. Markham (U.S.A.) via D. G. H. Edward	1953	Type-species: *M. gallisepticum*. Edward & Kanarek (1960)
S 6	Brain of turkey with torticollis and sinusitis	D. V. Zander via H. E. Adler (California)	1954	Adler & Yamamoto (1956), Zander (1961)
F 293	Trachea of chicken with 'C.R.D.'	D. V. Zander via H. E. Adler (California) J. Taylor via J. Fabricant (Cornell)	1956 1956	Adler & Yamamoto (1956) Calnek & Levine (1957)
SV	Turkey sinus exudate	H. E. Adler (California)	1957	Adler & Yamamoto (1957)
D	Turkey sinusitis exudate	J. E. Fahey via J. F. Crawley (Canada)	1953	Fahey (1954); Fahey & Crawley (1954). Called 'Crawley' by Chu & Newnham (1959)
BLOK	Air-sacs of chicken with 'C.R.D.'	M. E. Stumpel (Holland)	1958	Stumpel (1959)
'T'	Probably an egg contaminant isolated while passaging human NGU material	M. C. Shepard (U.S.A.) via E. Klieneberger-Nobel	1956	Shepard (1958); E. Klieneberger-Nobel (1962); R. Lemcke (1964)

Table 4. *Strains of classified and unclassified mycoplasma*

Name of strain	Serological group	Source	Origin	Date of origin	Remarks and references
Iowa 695	'9th' avian serotype	Air-sacs of 'pipped' turkey embryos	H. W. Yoder and M. S. Hofstad (Iowa)	1962	Agglutinates avian red cells. Yoder & Hofstad (1962)
A 36	Corresponding to Kleckner's group D	Trachea of chicken with primary infectious bronchitis (IBV)	H. P. Chu (Cambridge)	1955	'Fried-egg' type of colony. Chu & Newnham 1959)
A 326	Corresponding to Kleckner's group C	Sinus of chicken also containing *M. gallisepticum*	A. G. Newnham (Cambridge)	1958	'Fried-egg' type of colony
A 564	Corresponding to Kleckner's group C	Air-sacs of chicken also containing *M. gallisepticum*	A. G. Newnham (Cambridge)	1958	
Tu	Kleckner's group C (Kleckner, 1960)	Turbinates of 'normal' chicken	H. E. Adler (California)	1956	Non-pathogenic; Group II of Adler. (Ann. Meeting Amer. Vet. Med. Ass., Cleveland, Ohio, 1956–57)
Fowl	*M. gallinarum* corresponding to Kleckner's group B	Trachea of chicken with primary fowl pox	H. P. Chu (Cambridge)	1953	Non-pathogenic. Chu (1954); Edward (1954); PG. 16 (Edward & Freundt, 1956)
B 733		Trachea of chicken with mild coryza and fowl pox	F. T. W. Jordan (Liverpool)	1959	'Fried-egg-type of colony
A 64179		Trachea of 'normal' chicken	Y. V. Pereira (Connecticut)	1956	'Fried-egg' type of colony. Chu & Newnham (1959)
Laidlaw	*M. laidlawii*	Sewage	W. J. Elford and P. P. Laidlaw via L. Dienes (Boston)	1936	Saprophytic. Laidlaw & Elford (1936)
TC 7277	*M. laidlawii*	Tissue culture contaminant	E. S. Murray via L. Dienes (Boston)	1955–57	Isolated by Murray *et al.* (1957) from human conjunctival cell cultures. (Personal communication L. Dienes)
Bovine 'K'	*M. mycoides* var. *mycoides*	Contagious bovine pleuro-pneumonia	Type-culture (Colindale)	Unknown	Serologically identical with strain 403 (Hudson, Melbourne) and Bovine 'PI'
Bovine 'PI'	*M. mycoides* var. *mycoides*		Type-culture (Pasteur Institute)	Unknown	Classified as PG. 1 by Edward & Freundt (1956)
G 1/61	Unnamed	Lung of goat with pleuro-pneumonia	C. P. Pillai (Khartoum)	1961	Serologically indistinguishable from *M. mycoides* var. *mycoides*. Cottew & Hudson (personal communication), Lemcke (1964)
Goat	*M. mycoides* var. *capri*	Pleural fluid of goat with pleuro-pneumonia	H. P. Chu and W.I.B. Beveridge (Ankara and Cambridge)	1950	Edward (1953; 1954); classified as PG 3 by Edward & Freundt (1956)
Agalactiae	Corresponding to *M. agalactiae*	Infected goat's milk from V. Zavagli (Italy)	E. Klieneberger-Nobel (Lister Institute)	1953	Lemcke (1964)
2098/61	Corresponding to *M. pulmonis*	Rat lung pneumonia	I. Brewer & D. E. Stevenson (Tunstall Laboratory, Sittingbourne)	1961	Serologically indistinguishable from L 3 (Klieneberger, 1938)

Nitrofurazone
Neoarsphenamine (Neosalvarsan)
Nystatin (Mycostatin)
Polymixin B sulphate (Aerosporin)
Sodium aurothiomalate (Myocrisin)

Experimental method

Two drops of a 2- to 3-day broth culture of each strain were inoculated into a series of tubes containing 2 ml. of broth and falling concentrations of the drug under test. When the drugs were water-soluble, the concentrations were 1000, 200, 40, 8, 2, 0·5, 0·1, 0·02 and 0·004 μg./ml. broth. A broth control without drugs was included in the series. When testing against Furazolidone, Nitrofurazone and antrycide, the concentrations lay between 200 and 0·004 μg./ml., while with prothidium bromide the concentrations lay between 40 and 0·004 μg./ml. Erythromycin and antrycide were dissolved in a little methanol before adding to the broth, and Furazolidone was similarly dissolved in a little dimethylformamide. Concentrations of nystatin and polymixin are given in units/ml., the concentrations of nystatin lying between 200 and 0·004 units/ml. The solutions were made up just before the tests.

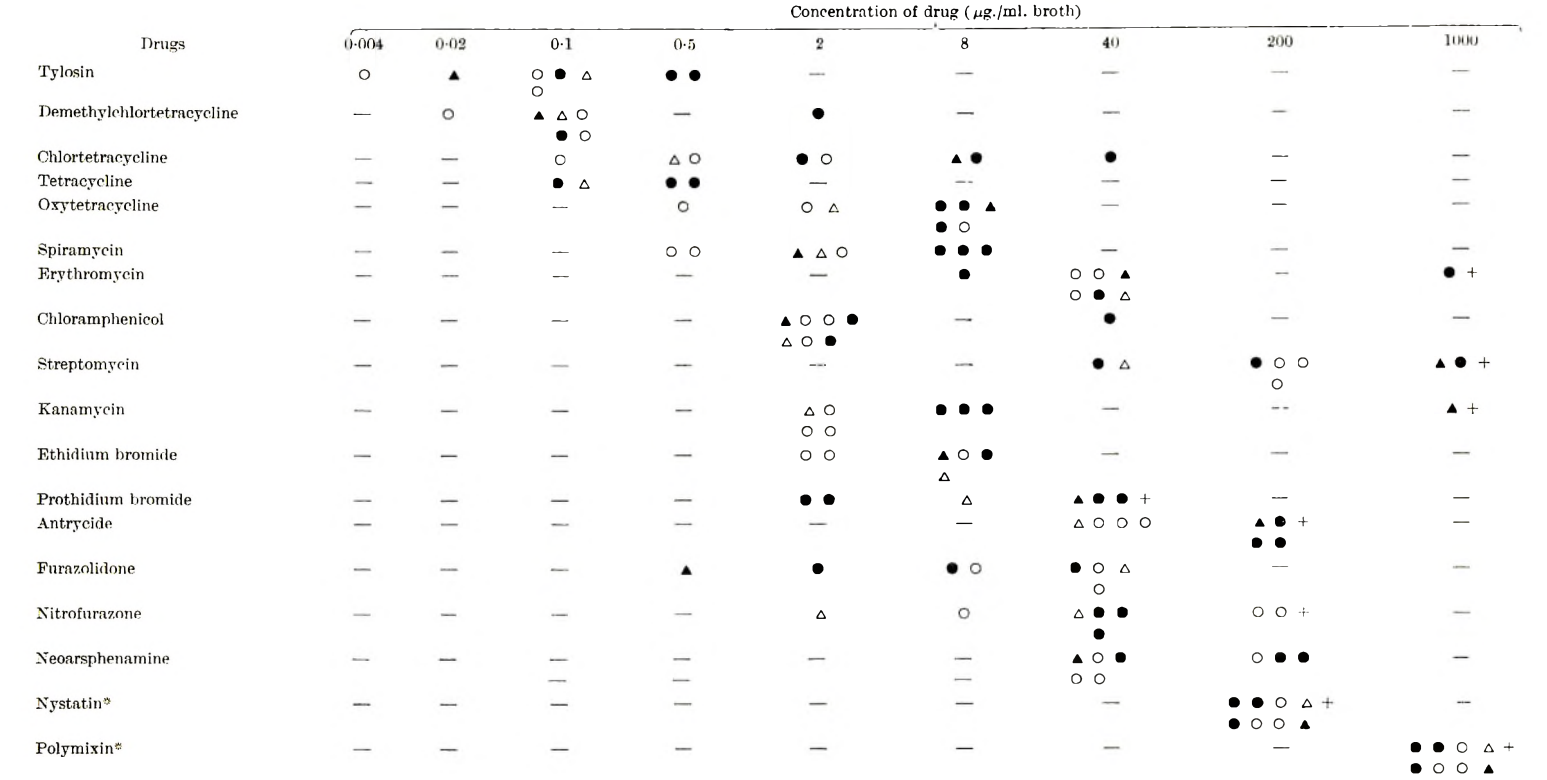
After inoculation the tubes were incubated at 37° C. for 7 days and a record kept of acid production as indicated by Andrade's indicator which was used as an index of growth. At the end of 1 week a loopful from the tube containing the highest concentration of the drug to show acid production was plated on to agar and the plates incubated for 2–4 days, after which they were examined for colonies by means of a dissecting microscope of $\times 35$ magnification and using oblique, transmitted light. Many of the tests were repeated at least once and results varying by more than one tube were rarely encountered. *M. agalactiae* and the three strains of *M. gallinarum* differed from the rest by not fermenting glucose; with these organisms growth was estimated by plating from each tube on to solid medium from the second day onwards.

RESULTS

The sensitivities *in vitro* of the thirty-six strains of *Mycoplasma* and the L-form of *Streptobacillus moniliformis* in liquid medium are presented diagrammatically in Tables 5–7.

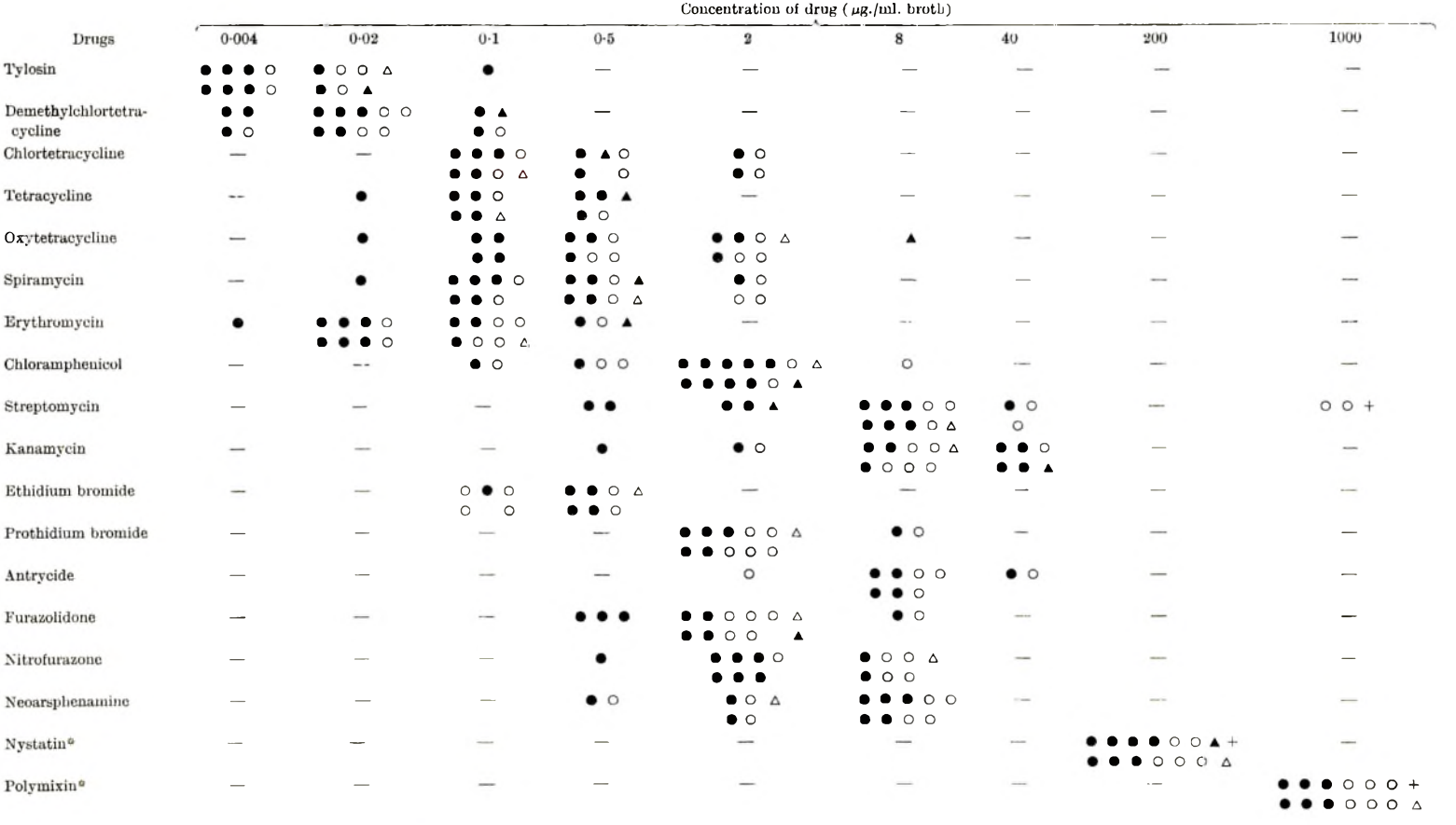
The results obtained with sodium aurothiomalate (Myocrisin) are not included in the tables owing to the frequent occurrence of a zone of inhibition with growth at both higher and lower drug concentrations when testing strains of *M. gallisepticum*. Thirteen strains of *M. gallisepticum* were tested repeatedly, some in triplicate on the same day and some on different days (to a maximum of five occasions). Variable results were obtained—sometimes no 'zoning' occurred and the strains grew in all concentrations from 0·004 to 1000 μg./ml.; sometimes the maximum concentrations permitting growth varied from 0·004 to 40 μg./ml.; but on most occasions, from about the fourth day onwards, acid production was

Table 5. *Sensitivities* in vitro *of avian mycoplasmata other than* Mycoplasma gallisepticum *to drugs*



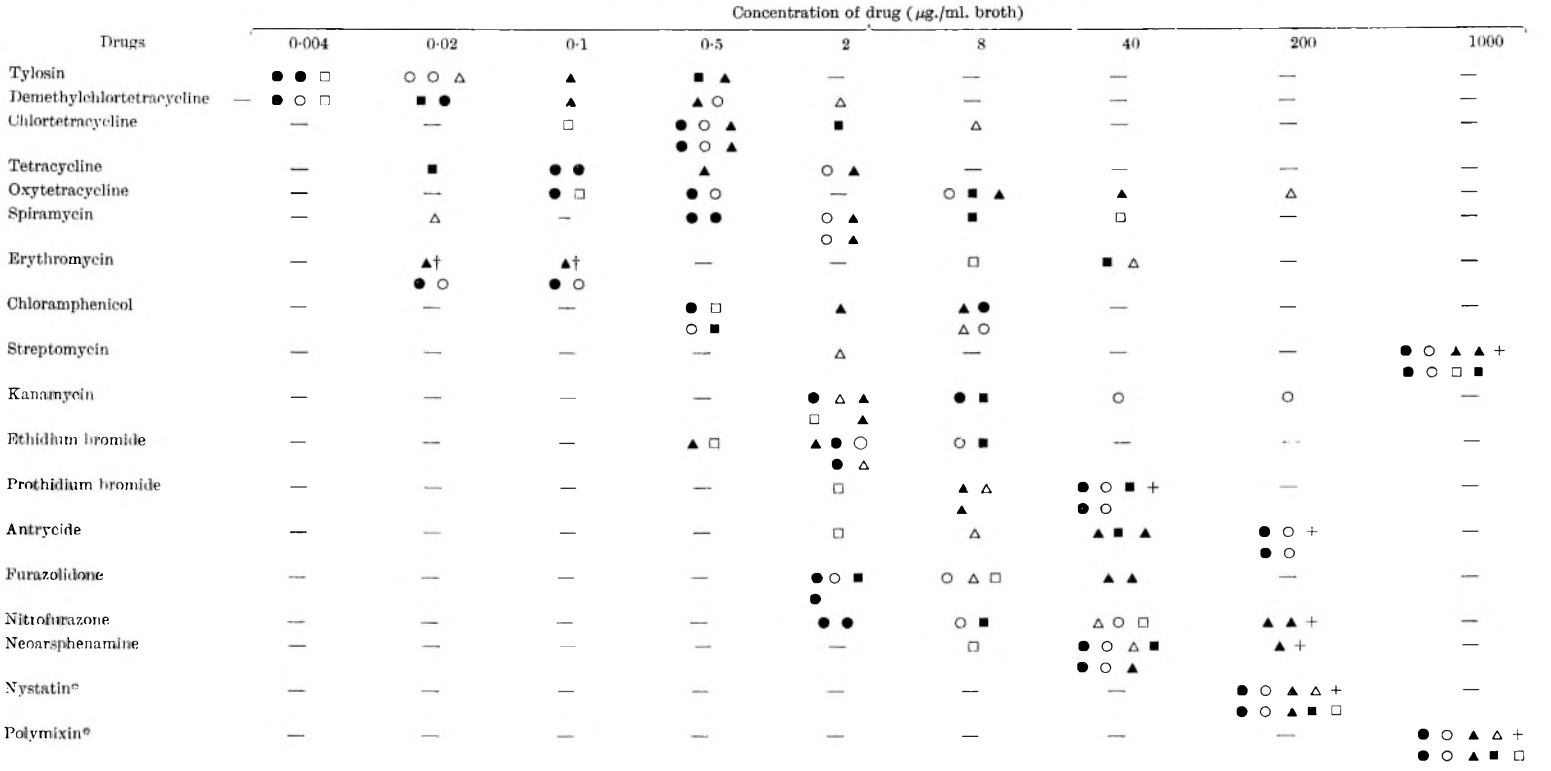
Key. Each symbol represents a single strain of mycoplasma; its position indicates the maximum concentration of drug permitting its growth. ●, 'Fowl', B 733; A 64179 (*M. gallinarum*; Kleckner's Group B); ○, TU, A 326, A 564 (Kleckner's group C); △, A 36 (Kleckner's group D); ▲, Iowa, 695; +, Highest concentrations tested; * concentrations in units/ml.

Table 6. *Sensitivities* in vitro *of* Mycoplasma gallisepticum *to drugs*



Key. Each symbol represents a single strain of mycoplasma; its position indicates the maximum concentration of drug permitting its growth. ●, British strains; ○, North American strains; ▲, Dutch strain; △, 'T' strain; +, highest concentration tested; *, concentrations in units/ml.

Table 7. *Sensitivities* in vitro *of L* 1 *and mammalian and saprophytic mycoplasmata to drugs*



Key. Each symbol represents a single strain of mycoplasma; its position indicates the maximum concentration of drug permitting its growth. ●, strains from bovine pleuropneumonia; ■, *M. agalactiae*; ▲, saprophytic strains; ○, strains from caprine pleuropneumonia; □, rodent strain (2098/61); △, L 1: L-form. *, concentrations in units/ml.; †, see text; +, highest concentration tested.

observed in concentrations of 0·004 and 0·02 μg./ml., and again at 8 μg./ml., with a zone of inhibition lying between 0·1 and 2 μg./ml., and then from 40 to 1000 μg./ml. This inhibition might remain for the whole 7 days (or longer), or only one tube would finally show inhibition, or 'zoning' would have disappeared altogether by the end of the test period.

This phenomenon was not observed when testing the other strains of *Mycoplasma* against sodium aurothiomalate. The maximum concentration permitting growth of avian non-pathogenic strains varied from 2 to 200 μg./ml., while Iowa 695, the two saprophytic strains, the rodent strains and the two goat pleuropneumonia strains grew in all concentrations up to 1000 μg./ml. *M. agalactiae* and Bovine 'PI', however, did not grow in over 40 μg./ml., and Bovine 'K' was inhibited by 8 μg./ml.

DISCUSSION

Differing techniques, different media, the use of solid or liquid medium, the decrease in activity of some drugs in solution over different test periods, the comparison of different species and of different strains within a species, and the ready emergence of resistant strains, could all contribute to the varied results obtained by independent workers.

It was because of the wide differences observed between the sensitivity to erythromycin of human genito-urinary mycoplasmata and strains of *M. gallisepticum*, both *in vitro* and *in vivo*, that this comparative study of drug sensitivities was initiated. Reports from all workers studying human strains stressed the almost complete lack of sensitivity of the strains to the drug *in vitro* (Keller & Morton, 1953; Harkness & Bushby, 1954; Blyth, 1958) and *in vivo* (Rubin, Somerson, Smith & Morton, 1954). Carski and Shepard (1961) also reported the insensitivity of their tissue culture contaminant (? human) strains to 15 μg./ml. of the drug.

A few workers have found erythromycin sensitivities of other mammalian mycoplasmata which compare well with those of human urethritis strains, although there are exceptions (see Table 1). These findings contrasted with reports of high sensitivity, both *in vitro* and *in ovo*, of most pathogenic avian mycoplasmata (see Tables 1 and 2). Inglis (pers. comm.) observed variations in sensitivity of strain A 514 of from 0·125 to 1·0 μg./ml. after 7 days' incubation, depending on the concentration of organisms in the inoculum.

In our experiments, twenty strains of *M. gallisepticum* were inhibited by 2 μg./ml. or less of erythromycin, the maximum concentration permitting growth varying between 0·004 and 0·5 μg./ml. The non-pathogenic avian strains, however, were capable of growth in 8 to 1000 μg./ml. *M. agalactiae* and 2098/61 were also relatively insensitive.

It will, however, be seen that the results obtained with the two saprophytic strains resemble those with *M. gallisepticum*, and an unusual result was observed with the goat and bovine pleuropneumonia strains. After 5 days' incubation the maximum concentrations permitting growth were recorded as lying between 0·004 and 0·5 μg./ml. for Bovine 'K' and G 1/61, and between 0·02 and 2 μg./ml. for

Bovine 'PI' and 'goat'. After 7 days, however, growth of all four strains had occurred up to much higher concentrations—8–1000 μg./ml. This result was probably due to the loss of activity of the drug after 5 days, the drug being bacteriostatic against these strains, but bactericidal against all other strains tested. Compared with the tetracyclines, however, erythromycin seems in general to be more stable in solution. Unlike chlortetracycline, its inhibitory action increases with increased alkalinity (Haight & Finland, 1952*a*). Blyth (1958) studied in detail the loss of activity of erythromycin, neomycin, tetracycline, oxytetracycline, chloramphenicol, spiramycin, streptomycin and some other drugs over a test period of 5 days. Although erythromycin (and neomycin) were the only two drugs whose action had not decreased over 5 days in agar at 37° C., Haight & Finland (1952*a*) reported a progressive deterioration in activity of all solutions of the drug in broth over 4–7 days at 37° C. and at room temperature. They also stated (Haight & Finland, 1952*b*) that the drug exerted its effect best on multiplying bacteria and that its action could be either bacteriostatic or bactericidal depending on the sensitivity of the organisms concerned and the concentration of the antibiotic.

Tetracycline, together with the derivative forms of this antibiotic, have been tested extensively against mycoplasmata *in vitro* and *in vivo*. In our experiments it inhibited thirteen strains of *M. gallisepticum* at concentrations varying from $< 0{\cdot}1$ to < 2 μg./ml., and similar concentrations were recorded for most of the other mycoplasmata. This compares favourably with the findings of most other workers (see Table 1). Wide variations found by Yamamoto & Adler (1956) when testing ten different avian strains, and by Domermuth (1958), were probably due to selection of resistant organisms, as Blyth (1958) later reported that the M.I.C. for tetracycline against his human mycoplasmata increased from 0·5 to 16 μg./ml. over twenty subcultures in the presence of the drug.

Selection of resistant strains and loss of activity of the drug is of special significance when examining the effect of tetracyclines on growing organisms. This is particularly true in the case of chlortetracycline, which is the least stable of the tetracyclines particularly in solution at incubator temperatures, in an alkaline pH (Lepper, 1956) or when in contact with serum or ascitic fluid (Paine, Collins & Finland, 1948*b*). At pH 2·5 the half-life of chlortetracycline is about 14 days, while at pH 8·5 it is only about 4 hr. Thus perhaps also in our experiments a false picture is given, where, if the test period had been shorter, the figures presented would have been somewhat lower. Blyth (1958) exposed his mycoplasmata to the drug for only 2 days, but found that the activity of chlortetracycline had greatly decreased after only 1 day's incubation at 37° C. The variation in results obtained with different avian mycoplasmata might also have been partly due to prolonged tetracycline therapy of infected birds before isolation of the organism again resulting in emergence of resistant strains (Fahey, 1957; Osborn & Pomeroy, 1958; Osborn, Mataney & Pomeroy, 1960; Newnham, 1963).

Figures given by other authors for the inhibition of mammalian mycoplasmata were considerably higher than those recorded in our experiments (see Table 1). The same was true of *M. gallisepticum*, where our seventeen strains were inhibited

at concentrations between 0·1 and 8 μg/ml., figures, in general, somewhat lower than those reported by previous workers, although Gross (1961) did comment that the drug would have long since been inactivated over his test period of 4 weeks.

Considerable variations in sensitivity were obtained by all workers with oxytetracycline. It was one of the first antibiotics to be tested, with favourable results *in vitro* and *in vivo*, against human genito-urinary mycoplasmata and non-gonococcal urethritis. Robinson, Wichelhausen & Brown (1952), testing twenty-eight strains from human rheumatic and genito-urinary diseases, observed that more than half of their strains were completely inhibited by 1·0 μg/ml. or less. They commented that this drug was superior to chloramphenicol, chlortetracycline, streptomycin and sodium aurothiomalate, perhaps because of its greater stability, although there was apparently some loss of activity over the test period. They noted great differences between the minimal inhibitory concentrations of the drug and the minimal lethal concentrations for most strains, sometimes as great as 32-fold or occasionally 256-fold.

Results obtained by other workers with oxytetracycline against mammalian mycoplasmata seem, in general, to indicate a lesser sensitivity than the human strains (see Table 1), but in our experiments inhibition was obtained at quite low concentrations of the drug. Strains of *M. gallisepticum* were generally inhibited at an even lower concentration than were the other mycoplasmata (0·1–8 μg./ml.), figures which compare favourably with those of most other workers.

The results with demethylchlortetracycline in our experiments showed that its activity against most of the thirty-three mycoplasma strains was higher than that of the other tetracyclines. All strains except one were completely inhibited at concentrations ranging from less than 0·02 to 2 μg./ml. The apparent superiority of this tetracycline may have been due to its much greater stability in solution over 7 days at 37° C. (Finland & Garrod, 1960).

Chloramphenicol, a broad-spectrum antibiotic commonly used in the past against mycoplasma infections, was tested but was found somewhat less inhibitory than the tetracyclines. Again very varied results were obtained by previous workers. Maximum concentrations of the drug permitting growth of our strains ranged from 0·5 to 40 μg./ml. although nineteen strains of *M. gallisepticum* were inhibited between 2 and 8 μg./ml.

The results obtained with streptomycin differed widely, not only between the *M. gallisepticum* group and the other mycoplasmata, but also amongst the strains of *M. gallisepticum* themselves. The majority of the heterogeneous group were comparatively insensitive to the drug and grew in concentrations of from 40 to 1000 μg./ml. Two strains of *M. gallisepticum*, however, both from North America, were also able to grow in 1000 μg./ml. and three strains (from Britain and the U.S.A.) were capable of growth in 40 μg./ml. It is perhaps worth noting that the British strains were in general more sensitive to the drug than those from the U.S.A. and Canada, where streptomycin may have been more widely used in the past for treatment of avian respiratory mycoplasmosis.

The sensitivities reported here were comparable with those of previous workers

who also found great variability depending on the origin of the strains concerned, and on whether the minimal inhibitory or minimal lethal concentrations of the drug were recorded. As with our findings, the pathogenic avian strains were in general more sensitive to streptomycin than the human, mammalian and tissue culture strains tested.

With streptomycin the problem of very rapid 'one-step' resistance must be considered (Blyth, 1958; Domermuth, 1960). According to the work of Blyth, using human genito-urinary strains, this resistance was permanent and remained after twenty-seven passages in drug-free medium.

Spiramycin at low concentrations was effective in inhibiting many strains of *Mycoplasma* in these experiments and, in particular, twenty strains of *M. gallisepticum* which were inhibited at concentrations between 0·02 and 8 μg./ml. This is comparable with the findings of Inglis (pers. comm.), who, using a test period of 7 days in broth, reported the M.I.C. of strain A 514 as 0·125–4 μg./ml., depending on the number of organisms in the inoculum. He reported a similar relative range of activity (0·008–0·125 μg./ml.) for tylosin against strain A 514. Of the nineteen drugs tested in our experiments, tylosin appeared to be the most active under our test conditions. The drug prevented growth of fifteen out of sixteen strains of *M. gallisepticum* at concentrations between 0·02 and 0·1 μg./ml., findings similar to those of Inglis.

Few workers have tested tylosin against mycoplasmata isolated from disease in mammals. Pak (pers. comm.), in Turkey, however, found a minimal inhibitory concentration of 0·5–1·0 μg./ml. when testing two goat pleuropneumonia strains, and Hudson (pers. comm.) in Australia found that the bacteriostatic dose of tylosin against two strains from bovine pleuropneumonia was 0·07 μg./ml. Promising results with *in vivo* work have been reported by a few workers on avian and mammalian mycoplasmal diseases, but further investigation is necessary to determine the true efficacy of this drug *in vivo* after encouraging *in vitro* results.

Kanamycin has been widely used against mycoplasma contamination of tissue cultures. Successful eradication has been reported by most workers (see Table 1), but concentrations of the drug and application time have varied considerably. Emergence of resistant strains has apparently not yet become a problem, although Gourevitch *et al.* (1958*b*) were able to produce resistant strains of bacteria without difficulty.

Although not very active in comparison with the other commoner antibiotics, in our experiments kanamycin had a range of activity of 2–200 μg./ml. against all thirty-four strains tested. Only two strains ('goat' and Iowa 695) were capable of growth in 200 and 1000 μg./ml. respectively. L 1 was also completely inhibited at 40 μg./ml.

Kanamycin has advantages over the earlier antibiotics (penicillin, streptomycin, tetracycline, erythromycin, etc.) in that it is active against organisms which have become resistant to the other antibiotics, although a slight incomplete cross-resistance was found with neomycin and streptomycin by Gourevitch, Hunt & Lein (1958*a*). Gourevitch *et al.* (1958*b*) stated that at sufficiently high concentrations this antibiotic was bactericidal; this concentration being twice the bacterio-

static concentration against *Staphylococcus aureus*. Its main advantage when used against *Mycoplasma*, however, is in tissue culture work, where kanamycin can be used at very high concentrations (up to 400 μg./ml.) without detrimental effect on the tissue culture cell-systems themselves (Pollock, Kenny & Syverton 1960; Smith, Lummis & Grady, 1959).

In vitro results obtained in our experiments and in those of previous workers showed that both Furazolidone and Nitrofurazone had a somewhat greater activity against *M. gallisepticum* than against the other species of *Mycoplasma* tested. The maximum concentrations of Nitrofurazone and Furazolidone permitting growth of *M. gallisepticum* varied between 0·5 and 8 μg./ml., concentrations similar to those reported by Gross (1961). Domermuth & Johnson (1955) and Domermuth (1958) found considerable differences between the minimal inhibitory and minimal lethal concentrations of Furazolidone against two pathogenic avian strains (A 5967 and Winchester), the M.I.C. varying between 0·1 and 10 μg./ml., and the M.L.C. being 10 μg./ml. for both strains.

In contrast to these findings, most of the strains other than *M. gallisepticum* were still capable of growth in concentrations of 0·5 to more than 200 μg./ml. of Furazolidone and 2 to more than 200 μg./ml. of Nitrofurazone.

Ethidium bromide, prothidium bromide and antrycide have for some years been used in the treatment of bovine trypanosomiasis in African countries, but they have not been commonly used against mycoplasmal diseases. The only previous work on the action of any of these drugs *in vitro* on organisms of the mycoplasma group has been reported by Nasri (1963). Using Dafaalla's medium (Dafaalla, 1961) he tested four strains of *M. mycoides* var. *mycoides* against ethidium bromide and found that the drug had a bactericidal effect only at 1000 μg./ml. after 6–24 hr. exposure. This is in contrast to our findings, where the two strains of *M. mycoides* var. *mycoides* and G 1/61 were inhibited at between 2 and 8 μg./ml. *M. mycoides* var. *capri* and *M. agalactiae* were both inhibited at between 8 and 40 μg./ml., and 2098/61 was inhibited by 0·5–2 μg./ml. The results obtained with mammalian mycoplasmata were very similar to those found with the non-pathogenic avian mycoplasmata, although twelve strains of *M. gallisepticum* were considerably more sensitive, all being inhibited at between 0·1 and 2 μg./ml.

Results obtained with prothidium bromide and antrycide also showed that strains of *M. gallisepticum* were generally more sensitive than the other strains of *Mycoplasma*. Antrycide appeared to have very little inhibitory effect on the mammalian or non-pathogenic avian strains, most strains being capable of growth in 40–200 μg./ml. Nine out of eleven strains of *M. gallisepticum*, however, were inhibited between 8 and 40 μg./ml. Figures obtained with prothidium bromide lay, in general, between those obtained with ethidium bromide and antrycide, *M. gallisepticum* again being rather more sensitive than the other strains of *Mycoplasma*.

Neoarsphenamine and other polyvalent organic arsenicals have been tested against mycoplasmata *in vitro*, *in ovo* and *in vivo*, sometimes with considerable effect. In our experiments neoarsphenamine was distinctly more active against the sixteen strains of *M. gallisepticum* than against the group of heterogeneous mycoplasmata.

The maximum concentrations of drug permitting growth of the former group varied from 0·5 to 8 μg./ml., whereas for the latter group figures of 8–200 μg./ml. were obtained. Turner (1960) found that the V 5 strain of *M. mycoides* var. *mycoides* was inhibited by 125 μg./ml. neoarsphenamine or 62·5 μg./ml. oxyarsphenamine, figures comparable with those we obtained against two similar bovine strains, where growth was completely inhibited between 40 and 200 μg./ml. Turner regarded the lack of sensitivity to organic arsenicals as unexpected for they are known to be superior to inorganic arsenicals as bacteriostatic agents.

The 'Zone Phenomenon' found when testing sodium aurothiomalate against *M. gallisepticum* in our experiments was also observed by Robinson *et al.* (1952), although they were testing human and rodent strains. They reported minimal lethal concentrations of the drug as 16–128 μg./ml., and commented that, unlike the tetracyclines, the inhibitory and lethal concentrations of this drug did not lie far apart. In our experiments with strains other than *M. gallisepticum*, very varied results were obtained, some being comparable with those of the above authors.

Explanations for the 'Zone Phenomenon' are not readily forthcoming. It is possible that a complex is formed by sodium aurothiomalate with constituents of the culture medium, which, at certain concentrations, is inhibitory for some mycoplasmata (Nineham, pers. comm.). The varying results obtained with the same strains on different occasions might then be due to different batches of medium and, in particular, to horse serum from different horses.

Polymixin allowed the growth of L 1 and 29 strains of *Mycoplasma* tested in a concentration as high as 1000 units/ml. (approximately 167 μg./ml.). No difference in sensitivity was observed between the *M. gallisepticum* group and the heterogeneous group. These findings were similar to those of Hatch (1949), who found that polymixin at 50 μg./ml. was ineffective against eight strains of human and rodent *Mycoplasma*. Carski & Shepard (1961) also found that their seven tissue culture strains were insensitive to the drug, and Wong & James (1953) reported the lack of inhibition by polymixin of a few strains of *M. gallisepticum* in chick embryos.

The suggested mode of action of polymixin has been reviewed by Newton (1956). He, and other workers, used Gram-positive and Gram-negative bacteria as test organisms. It is thought that this drug acts primarily on the protoplast membrane and/or cell wall by combining with the phospholipid components and this results in the disorganization of the osmotic barrier (Gale, 1963). As mycoplasmata do not possess the normal type of cell wall, it is perhaps not surprising to find that this drug exerts no inhibitory action on the strains tested.

When tested against the fungicide, nystatin, all thirty-one mycoplasma strains and L 1 grew actively in concentrations up to 200 units/ml. This is equivalent to approximately 66·7 μg./ml. Razin (1963*b*). using strains *M. laidlawii*, *M. mycoides* var. *mycoides*, *M. mycoides* var. *capri* and *M. gallisepticum*, also found no inhibition up to 125 μg./ml. over a test period of 48 hr. This test period was superior to ours in that nystatin is known to lose approximately 40–50% of its antifungal activity in 5 days when in organic solvent–water preparations, even at room temperature.

Similar lack of activity was reported by Lampen, Gill, Arnow & Magana-Plaza (1963). Using strain A 5969 of *M. gallisepticum* they found that growth was not inhibited over 5 days up to a concentration of 100 μg./ml.

Both sets of workers none the less found that the mycoplasmata absorbed a considerable quantity of nystatin. These results were unexpected in the light of previous findings that nystatin-sensitive fungal cells and protoplasts bound considerable amounts of the drug while nystatin-resistant bacterial protoplasts and eubacteria failed to do so significantly (Lampen, Morgan, Slocum & Arnow, 1959; Lampen, Arnow, Borowska & Laskin, 1962; Kinsky, 1962). Eubacteria contain only traces of sterols, or none at all (Fiertel & Klein, 1959) whereas mycoplasmata, like the nystatin-sensitive fungi, algae, protozoa and animal cells (Lampen *et al.* 1962) contain, especially in the cell membrane, considerable quantities of cholesterol (Smith & Rothblat, 1962; Razin, 1963*a*). Thus Razin (1963*b*) suggested that the differences in capacity of various organisms to bind such polyene antibiotics does not account satisfactorily for the selective toxicity of the drug.

SUMMARY

A study was made in liquid medium over 7 days at 37° C. of the inhibitory action of nineteen antibacterial, antifungal and antiprotozoal drugs on twenty strains of *M. gallisepticum*, eight other avian mycoplasmata, six mammalian mycoplasmata, two saprophytic mycoplasmata and the L-form of *Streptobacillus moniliformis* (L–1).

The twenty strains of *M. gallisepticum* from Britain and other countries showed a similar range of drug sensitivity except where resistant strains were included. Tylosin and demethylchlortetracycline appeared to have the highest inhibitory action, followed by erythromycin, spiramycin, tetracycline, chlortetracycline, oxytetracycline and ethidium bromide. A 'Zone Phenomenon' frequently occurred with sodium aurothiomalate, inhibition often being observed between 0·1 and 2·0 μg./ml. Polymixin and nystatin had no inhibitory effect on the growth of any mycoplasmata tested. With the exception of erythromycin and streptomycin in some cases, the pattern of sensitivity observed with the mycoplasmata of diverse origin was similar to that of *M. gallisepticum*, most strains, however, being somewhat more resistant than *M. gallisepticum* to many of the drugs.

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Hospital infections and hospital hygiene at Malmö General Hospital

1. The incidence of staphylococcal infections during three years

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The problems of hospital infections have been reviewed most thoroughly by Williams, Blowers, Garrod & Shooter (1960) and the current views on the epidemiology and prevention of such infections were recently discussed at an international symposium in London (Williams & Shooter, 1963). Among those who have had an active interest in this field, there is general agreement on some basic principles regarding the control of hospital infections. At many large hospitals Infection Control Committees have been established. There are, however, comparatively few reports (Howe, 1956; Hitchcock *et al.* 1958; Jensen, 1962) on the effects of a broadly designed preventive programme upon the incidence of hospital infections, and there are still many hospitals without any coherent hygiene programme other than the general instructions issued by official national authorities.

The primary object of this study is to assess the validity of the hygiene programme of our own hospital. We feel that our experience might be stimulating to others.

The first part of this work deals with the incidence of hospital infections and the changes in this incidence in the 3 years of active preventive work. In the second part (Juhlin & Ericson, 1965) the various hygienic measures will be discussed in the light of the results presented in the first part.

MATERIALS AND METHODS

At this hospital there is no continuous record of hospital infections. However, as persons with primary staphylococcal infections are usually treated as outpatients, the number of purulent infections with *Staphylococcus aureus* among inpatients may, with some limitations, be regarded as an expression of the frequency of hospital infections.

This investigation deals with the purulent staphylococcal lesions in eight departments in the years 1960–62. Examples of purulent lesions are furuncles, carbuncles, wound abscesses, decubital infections and septicaemia. Pulmonary and urinary infections and cases of otitis media have been excluded, on the assumption that they would largely have been acquired outside the hospital.

Only the identity of the first culture of a particular strain of *Staph. aureus* from each patient has been included in this study.

The departments which have been studied are: Long-Term Diseases, Internal Chest Diseases, Internal Medicine, Obstetrics and Gynaecology, General Surgery, Chest Surgery, Plastic Surgery and Orthopaedics. Interest in taking bacteriological specimens does not seem to have diminished in these departments during the 3 years under investigation. No great changes have been made in their architecture or organization.

Identification and classification of Staphylococcus aureus *strains*

Typing with bacteriophages has been performed according to the standard international method (Blair & Williams, 1961). For the identification of strains we have also used the antibiogram (disk sensitivity method, H. Ericsson (Ericsson, Högman & Wickman, 1954; Ericsson & Swartz-Malmberg, 1959), with a sulphonamide, benzyl penicillin, streptomycin, tetracyclin, chloramphenicol, erythromycin, kanamycin), and the Tween 80 esterase test (Sierra, 1957). In the course of typing, subcultures have been made on water-blue agar (Ericson, 1962) in order to disclose mixtures of strains.

The methods for primary isolation and typing have remained unchanged in the period under investigation.

For the purpose of this study, the various strains of *Staph. aureus* have been divided into three classes:

(1) '*Virginal*' *strains.* These were resistant to two at most of the antibiotics mentioned above. The results of phage typing were heterogenous, and there are no indications that any of these strains has been truly epidemic at this hospital.

(2) '*Multiple-resistant*' *strains.* These were resistant to more than two antibiotics and did not belong to the '80/81' group (see below). Like the strains in the '80/81' group nearly all of these strains have been epidemic (in some cases rather endemic) at this hospital. Most of them have been lysed by phages belonging to group III (e.g. 7/47/54/75), but a large proportion have been non-typable with phages at 1000 × RTD (Routine Test Dilution).

(3) '80/81' *strains.* On phage typing, these strains were lysed by phages belonging to the '80/81' group (Parker & Jevons, 1963). Most of these strains were resistant to sulphonamides, penicillin, and streptomycin. The strains which were lysed by the same phages, but were more sensitive to antibiotics, have been placed in the virginal group.

This classification was easily carried out without doubtful decisions. Its significance will be discussed below.

Estimation of the incidence of infection

The incidence of infection is usually described as a percentage of the number of admittances or operations. In this investigation we have used a different basis for the determination of this frequency. As the chief purpose of this study is to note any changes in incidence which may have occurred, and to correlate such changes with preventive hygienic measures, it has been important to exclude as far as possible the influence of non-hygienic factors upon the incidence of infection Such factors are: (1) the mean number of beds available during each period,

(2) the extent to which the beds have been occupied, and (3) the mean duration per patient of the period in hospital.

The number of admittances reflects all these factors. The number of infections may, crudely, be simply correlated with the first two factors: the bed capacity and occupancy rate. The effects of the third factor, however, are more complicated. We have preferred to neglect the influence of this factor by correlating the infection numbers during different periods only with the bed capacity and occupancy rate.

This has been done as follows. The number of infections in each department has been noted semi-annually. This number has been related to an occupancy rate of 100 % of the maximum mean number of beds available during any of the six half-year periods. For each department and each half-year, a multiplication factor has been calculated according to the following formula:

$$\frac{100 \times \text{maximum bed capacity}}{\text{occupancy rate} \times \text{bed capacity}}.$$

RESULTS

Two terms will be used in describing the incidence of infection:

The 'infection rate' is the absolute number of purulent infections in a department as a percentage of the number of patients cared for during a certain period of time.

The 'relative infection number' is the absolute number of infections in a department multiplied by the above-mentioned factor.

In Table 1, the annual infection rates in the 3 years under investigation are listed for the eight departments. The relative importance of staphylococcal infections is strikingly different amongst the eight departments. In one respect, however, all

Table 1. *Infection rates* (Staphylococcus aureus) *of eight departments* 1960–62

Departments	Annual infection rates (%)		
	1960	1961	1962
Long-term diseases	15·78	5·96	3·80
Internal chest diseases	11·17	8·67	2·15
Internal medicine	1·39	0·62	0·98
Obstetrics and gynaecology	0·48	0·26	0·29
General surgery	1·41	1·54	1·11
Chest surgery	10·49	10·76	4·07
Plastic surgery	7·04	10·12	6·53
Orthopaedics	4·69	4·08	3·99
All eight departments	2·27	1·76	1·46

departments are alike, in showing lower infection rates in 1962 than in 1960. If all these departments are treated as one unit, the infection rate shows a steady decline from 1960 to 1962. The reduction is 22·5 % between 1960 and 1961, and 17·0 % from 1961 to 1962. If the infection frequency is described in terms of relative infection numbers, the reduction is 19·6 % in the first period, and 21·4 % in the last period.

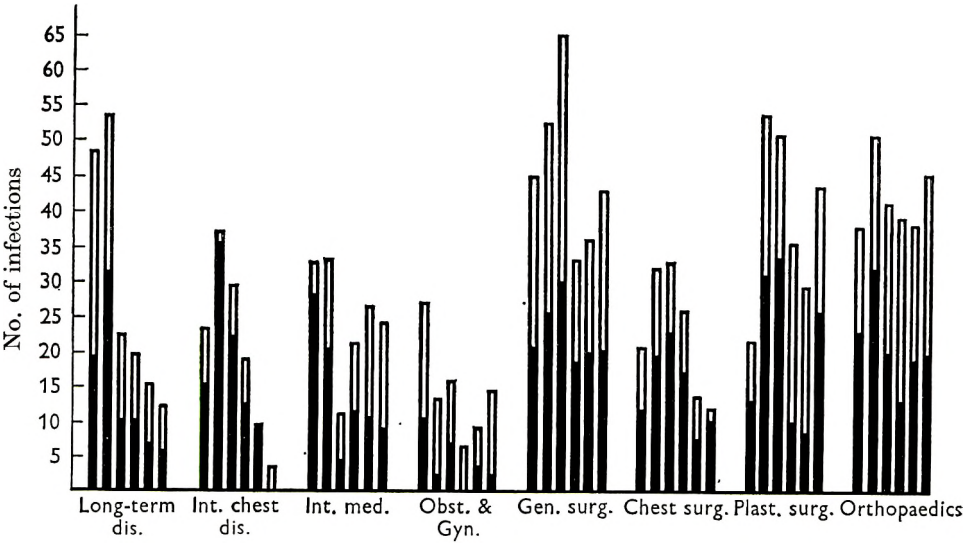

Fig. 1. Relative infection numbers of eight departments, semi-annually from 1. i. 1960 to 31. xii. 1962. For each department, every staple represents one half-year. The upper parts of the staples represent infections with virginal strains, and the filled lower parts infections with multiple-resistant strains and '80/81' strains.

Table 2. *Relative infection numbers of eight departments during two consecutive 18-month periods, and differences between the results from the two periods*

Departments	Classes of *Staph. aureus* strains	Relative infection numbers: 1. i. 60–30. vi. 61	Relative infection numbers: 1. vii. 61–31. xii. 62	Difference (%)
Long-term diseases	Virginal	63·6	23·5	−63·1
	Multiple-R + '80/81'	60·6	23·4	−61·4
	All	124·2	46·9	−62·2
Internal chest diseases	Virginal	16·6	9·9	−40·4
	Multiple-R + '80/81'	73·1	22·0	−69·9
	All	89·7	31·9	−64·4
Internal medicine	Virginal	24·1	40·8	+69·3
	Multiple-R + '80/81'	52·8	30·7	−41·9
	All	76·9	71·5	− 7·0
Obstetrics and gynaecology	Virginal	36·3	24·5	−32·5
	Multiple-R + '80/81'	19·7	5·9	−70·1
	All	56·0	30·4	−45·7
General surgery	Virginal	86·4	53·5	−38·3
	Multiple-R + '80/81'	75·9	58·5	−22·9
	All	162·3	111·8	−31·1
Chest surgery	Virginal	31·2	16·3	−47·8
	Multiple-R + '80/81'	54·2	34·8	−35·8
	All	85·4	51·1	−40·2
Plastic surgery	Virginal	48·8	64·8	+32·8
	Multiple-R + '80/81'	77·8	43·4	−43·9
	All	126·2	108·2	−14·3
Orthopaedics	Virginal	55·2	71·6	+29·7
	Multiple-R + '80/81'	73·8	50·5	−31·6
	All	129·0	122·1	− 5·3

In Fig. 1, the relative infection numbers of the eight departments have been calculated semi-annually. The top parts of the staples represent infections with 'virginal' strains, while the filled lower parts represent infections with '80/81' strains + 'multiple-resistant' strains. In all departments there is a marked drop in the relative infection numbers during the first or second half of 1961. This reduction is most pronounced amongst the infections with non-virginal strains.

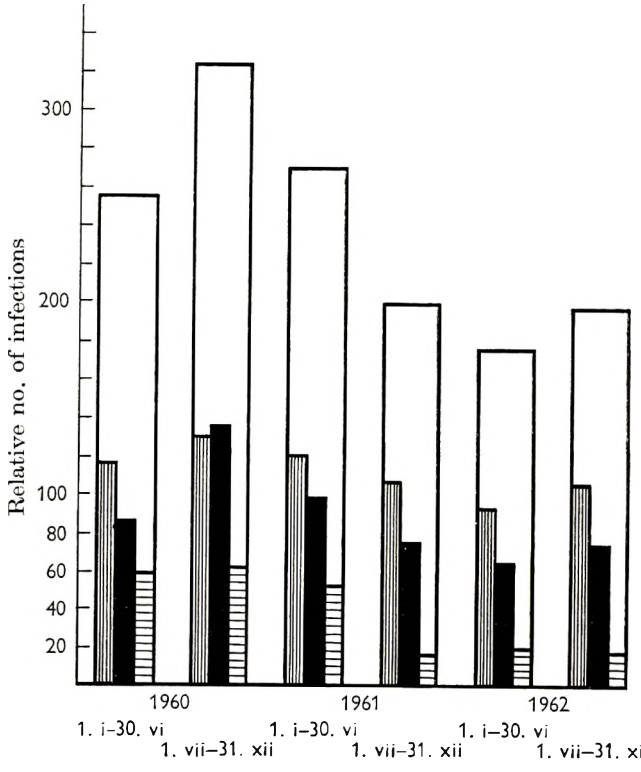

Fig. 2. Relative infection numbers of eight departments taken together. Semi-annual registration from 1. i. 1960 to 31. xii. 1962. Large staples: all infections with *Staph. aureus*. Small staples: Vertical stripes: infections with virginal strains. Black: infections with multiple-resistant strains. Horizontal stripes: infections with '80/81' strains.

In Fig. 2 this tendency may be seen even more clearly. Here, the semi-annual relative infection numbers have been summated, so that the eight departments are treated as one unit. The large staples represent all staphylococcal infections. Within each staple there are three smaller ones, which represent, from left to right, infections with 'virginal' strains, 'multiple-resistant' strains, and '80/81' strains. It is to be noted that the relative infection numbers during the last three half-yearly periods form a plateau that is lower than the corresponding numbers for any of the first three periods. From the small staples it is seen that the general reduction in the middle of 1961 was effected by a reduction in all kinds of staphylococcal infections, although the decrease in the number of infections with '80/81' strains is most evident.

In Table 2, the relative infection numbers for each department have been calculated for two periods each of 1·5 years. It is found that in every department the relative infection numbers for the second period are lower than those for the first period. In some departments there is an increase in infections with 'virginal' strains, but this is always compensated by a decrease in infections with 'non-virginal' strains.

Table 3. *Relative infection numbers of eight departments taken together during two consecutive* 18*-month periods, and differences between the results from the two periods*

Classes of *Staph. aureus* strains	Relative infection numbers 1. i. 60–30. vi. 61 (No.)	(%)	1. vii. 61–31. xii. 62 (No.)	(%)	Difference (%)
Virginal	362·2	42·6	304·7	53·1	−15·9
Multiple-R	318·7	37·5	212·4	37·0	−33·3
'80/81'	168·8	19·9	56·8	9·9	−66·4
All	849·7	—	573·9	—	−32·5

In Table 3, the corresponding figures are given for the eight departments treated as one unit, and the infections are divided into three categories with respect to the different classes of staphylococci. The proportion of each category of staphylococci found in the total sum of infections is also presented here. The reduction in infections with 'multiple-resistant' strains has kept pace with the general reduction in infections (about one-third), while the relative reduction of infections with '80/81' strains has been twice as great. In consequence, the proportion of infection caused by 'virginal' strains has increased.

DISCUSSION

The object of this investigation is to test the effectiveness of the hygienic measures which have been undertaken throughout the hospital. The material ought properly to include infections from departments which are representative of the various kinds of specialized modern hospital care, and on this basis, the departments of Paediatrics, Urology, and Infectious Diseases should also have been included. However, the first has been virtually free from staphylococcal hospital infections, the second has been reorganized during the period under investigation and, in the third department, special hygienic precautions make it unsuitable for comparison with the rest of the hospital.

If conclusions as to the efficiency of a general hygienic programme are to be drawn from the total extent of infection, a successful outcome must be demonstrated in at least a majority of the departments. It is evident from this investigation that, although proportions may vary considerably, all the departments had less staphylococcal infections during the second half of the 3-year period (Table 2).

It is seen from Table 1 and Fig. 1 that the reduction has been most pronounced and definite in departments with a high initial incidence of infection. Only one

department, Plastic Surgery, deviates from the low general plateau during the last three half-yearly periods. During the second half of 1962 a sharp rise occurred in the relative number of 'non-virginal' strains. It is impossible to state the cause of this, but the number of carriers of 'multiple-resistant' strains among the staff was unusually high at that time.

It is doubtful whether one should use mathematical statistics to examine the probability that the observed reduction is purely a chance phenomenon. In view of the multiplicity of factors which may have operated in these circumstances, we prefer to base an opinion on the epidemiological evidence presented.

If all departments are taken together, it is notable that the reduction in infections with 'virginal' strains is much less distinct than the reduction in infections with 'non-virginal' strains, especially with '80/81' strains. This may have several explanations.

The fact that the reduction of infections with 'virginal' strains has been smaller than the average is most easily explained on the assumption that this group includes a number of infections acquired outside the hospital.

Infections caused by patients' 'own' strains, but appearing within the hospital probably belong to this group, as well as infections with strains which have been transferred to the patients by droplet infection. The only serious attempt to block this route of infection has been the wearing of surgical masks by the operating staff.

Table 4. *The distribution of* Staphylococcus aureus *strains from various sources into different classes. Material collected in Malmö* 1960–62

	Virginal		Multiple-R		'80/81'		Total
Origin	No.	(%)	No.	(%)	No.	(%)	No.
Infections in in-patients	509	47·6	390	36·4	171	16·0	1070
Infections in out-patients	169	76·8	3	1·4	48	21·8	220
Carriers among hospital staff	167	81·9	30	14·7	7	3·4	204
Carriers outside the hospital	57	100·0	0	—	0	—	57

These assumptions are founded on several reports from other hospitals (Finland, Hirsch & Wallmark, 1960; Hinton & Orr, 1957; Koch, Lepley, Schroeder & Smith, 1959; Parker & Jevons, 1963; Rountree, 1963; Vogelsang & Haaland, 1959; Vogelsang & Iversen, 1959–60) and also on Winblad's studies at this hospital (Winblad, 1960*a*, *b*). These results agree with those of a number of recent investigations on various populations, both at this hospital and in the city of Malmö, summarized in Table 4. It may be noted that 'virginal' strains were dominant in all groups except in-patients, and that almost all 'multiple-resistant' strains occurred inside the hospital only. Strains belonging to the '80/81' group, however, were rather common also in infections among out-patients (although probably

not as common as it seems. Bacteriological examination is requested much more often in cases of furunculosis than in other types of out-patient infection).

There is a striking discrepancy between the distribution of strains carried by the hospital staff and the strains found in the patients' infections. However, it may be mentioned that 'non-virginal' strains were carried by the staff members in wards with high infection rates more often than in other wards.

As for the '80/81' strains, doctors and nursing staff have for many years been instructed to regard the occurrence of such strains within their departments as a danger signal. Patients infected with such strains have been isolated, when possible, and carriers of such strains have been treated with antibacterial nasal creams. This may explain why infection with this group of staphylococci has decreased more than the average. On the other hand, the special warnings for '80/81' strains had already been issued before 1960.

On the basis of this information, it seems reasonable to accept the assumption that there has occurred at this hospital a real reduction in the number of staphylococcal infections during the course of 1961, and that the causes of the reduction may be found amongst such preventive measures as limit the transmission and multiplication of staphylococci within the wards.

SUMMARY

The incidence of purulent staphylococcal infections at a large Swedish university hospital was followed during 3 years and has been described as an expression of the incidence of hospital infections.

A marked decrease in the relative number of infections was found. The decrease was most evident among infections with '80/81' strains and other antibiotic resistant hospital strains and was parallel in all the departments investigated. This is taken as an evidence for the assumption that the lowering of the amount of staphylococcal infections was brought about by a factor that was active throughout the hospital, probably some hygienic preventive measure.

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J. Hyg., Camb. (1965), **63**, 35
With 1 *plate*
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Hospital infections and hospital hygiene at Malmö General Hospital

2. Hygienic measures and their correlation with the incidence of infection

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In December 1958 an Infection Control Committee was set up at the Malmö General Hospital. This Committee had a threefold purpose: the investigation of hygienic conditions in the different departments, the drawing up of procedural rules which would apply to the entire hospital, and the introduction of improvements on the basis of certain economic calculations.

Previously it had been the practice for the head of each department, in consultation with his staff, to safeguard hygiene; as a result of this it became increasingly difficult for the Central Stores to supply all the different types of materials and liquids required.

A working committee was therefore set up within the Infection Control Committee, with representatives from the Purchasing Department, the Central Stores, the Planning Department, the Hospital Superintendent's Office, the Nurses' Instruction Section, together with the hospital Governor and two doctors from the Institute of Clinical Bacteriology. When required, members of other departments were also consulted.

The problems involved often called for fairly extensive experiments with respect to use, practicability and satisfactory results in relation to the economic implications. One of the most important aspects of the work was the investigation of available products, their prices and qualities. In certain cases entirely new products had to be designed. As the various problems were solved instructions were drawn up and printed for distribution to all relevant departments.

METHODS

Fig. 1 gives a 6-monthly review of the more important measures introduced, usually simultaneously, in all the departments. In order to exhibit these measures in relation to the incidence of infection, one of the departments has been selected as an example to demonstrate the results achieved. The year 1959 has also been included here.

In view of the relatively unqualified personnel who carry out a great deal of the work affected by the instructions, and in order to avoid major departures from the required practice, the instructions have been drawn up in considerable detail. See, for example, the specimen memorandum on thermometer disinfection (Fig. 2).

The dates when the different measures were introduced and their principal features are shown in the following paragraphs.

1959. 1 *January*–30 *June*

Memorandum on thermometer disinfection (see Fig. 2). This memorandum applied to all departments except the departments of Infectious Diseases and Paediatrics. Here primary disinfection of thermometers is carried out with a 5% chloramine solution instead of a 0·1% benzalkonium chloride solution.

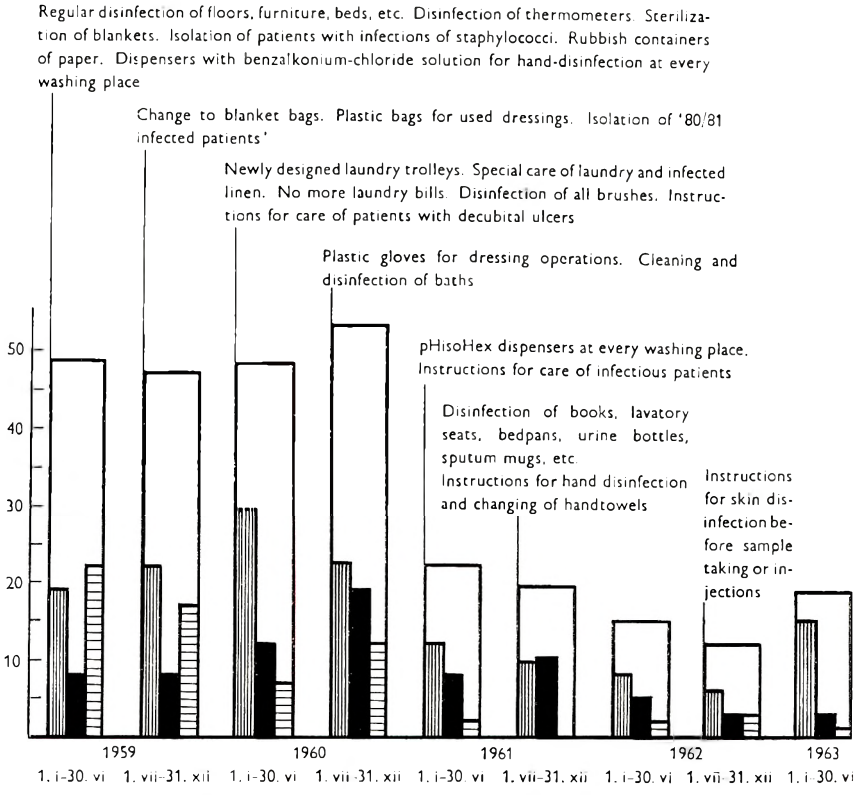

Fig. 1. Number of infections at the department of long-term diseases registered semi-annually, including information on the hygienic measures introduced during each 6-month period. □, Total number of infections; ▥, virginal strains; ■, multiple resistant strains; ▤, 80/81 strains.

Memorandum on floor cleaning and disinfection. All dry brushing, dry mopping and vacuum cleaning in the departments is forbidden. Daily cleaning with a scouring cloth and warm water; cleaning agents to be used only where required. Once a week: wiping off with a scouring cloth damped with a solution of 0·1% benzalkonium chloride. Once a month: a thorough clean with cleaning agents, followed by rinsing and floor polishing. Once a year: a major cleaning operation with thorough scrubbing of all floors.

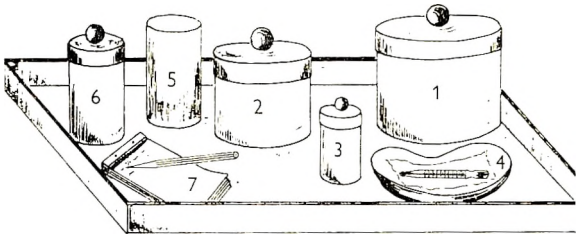

Fig. 2. Use of thermometers. Wards

1. Jar containing 0·1 % benzalkonium chloride solution. This solution is changed once daily. Used for storing *clean* thermometers.
2. Jar for 5 × 15 cm. wadding pads (suitable for length of thermometers).
3. Jar of exploration ointment, water soluble.
4. Round dish (number according to number of personnel engaged in temperature taking).
5. Jar containing 0·1 % benzalkonium chloride solution. This solution is changed each time the jar is used. Used for storing *used* thermometers.
6. Jar with lid, for used wadding.
7. Pencil and pad.

These utensils are kept in the rinsing-room, on a tray or on a shelf, or in a cupboard if so required.

Procedure

1. Place sheet of toilet paper in a round dish.
2. Take the required number of thermometers, dip the ends into exploration ointment and then place the thermometers in the round dish.
3. The dish with the thermometers, the wadding pads, the jar with benzalkonium chloride solution, the jar for used wadding pads and the pencil and block are taken to the ward.
4. The thermometers are distributed. The wadding pads are placed on the bedside stands and the patients are requested to lay the thermometers on the pads if they are removed before the nurses return.
5. The thermometers are dried off and read, after having been dipped if necessary into the benzalkonium chloride solution. The thermometers are then placed in the jar of benzalkonium chloride solution and the wadding pads into the jar with lid.
6. After at least 90 min. the thermometers are removed and washed mechanically under running, cold water. They are then replaced in the jar of benzalkonium chloride solution used for *clean* thermometers. Check that the mercury in all the thermometers is at minimum.
7. The two jars used for collecting used thermometers and used wadding pads are cleaned and boiled, or put into the autoclave, after each time they are used.

The cleaning of the thermometers should be the responsibility of one particular person.

This method of storing thermometers has two advantages: it is a satisfactory means of disinfection and it also saves time. GOVERNOR

Memorandum on bed cleaning

Daily wiping off of the metal parts of the bed, electric leads, the bed-side stand and other furniture (in cases of patients where there is a danger of infection a solution of 0·1 % benzalkonium chloride is used). Each time a new patient is to use a bed it is first disinfected with benzalkonium chloride. All mattresses must have a plastic cover. Mattresses may only be vacuum cleaned on balconies or elsewhere outside. All bedclothes and the laundry bags of infectious patients are treated with formalin before being laundered.

Paper waste-sacks, instead of the usual containers, to be used and the sack and its contents taken directly to the incinerator or refuse station.

Patients with open infections with *Staph. aureus* phage type '80/81' are isolated as completely as possible (due to the shortage of space this can only be carried out to a limited extent).

Benzalkonium chloride dispensers for hand disinfection (about 500) have been installed in the Departments of Long-Term Diseases and Infectious Diseases.

1959. 1 *July*–31 *December*

Introduction of plastic bags for collecting used dressings. Previously, used dressings were either put together with the patients' dirty laundry or were thrown into open bowls.

A rapid successive change-over to washable blanket bags in place of loose covers. Successive change-over from woollen blankets to cotton blankets, which can be autoclaved.

Special hygiene regulations for the hospital hairdresser.

1960. 1 *January*–30 *June*

Memorandum on the collection of dirty laundry and used dressings

Newly designed stackable trolleys, each carrying a sack for dirty laundry and a bucket with a plastic bag interior for used dressings, have been supplied to each department (Plate 1). Each bed team has a trolley with a sack sufficient for approximately five beds (15 kg.). The dirty sheets, etc., are not shaken, but are carefully folded and placed in the sack, which is properly tied up at the top when it has been filled. Laundry from infectious patients is collected in sacks marked with red tape and sent directly for disinfecting.

Dressings are applied or changed either before making up the beds or else no sooner than one hour afterwards. Dirty articles of clothing are placed in the laundry sack and used dressings in the plastic bag, which, in its turn, is put into the lidded bucket. When the bag is full, or the dressing operations completed, it is tied up at the top ready for incineration or removal to the refuse station.

Change of method for laundry disinfection

Instead of disinfection with formalin, infectious clothing, blankets, etc., are treated for 7 hr. in steam at 90° C.

Memorandum on the ointments tray

Utensils are kept in a dust-free cupboard. Ointments are applied with a wooden spatula, which is used only once. Hands should be washed between each patient, preferably with 70% (w/v) ethyl alcohol.

Memorandum on patients infected with staphylococci

These patients are isolated as thoroughly as possible. Staff use protective overall coats, which are kept in the room, disposable gloves, and wash their hands first in soap and water and then in a 0·1% benzalkonium chloride solution. Floors,

beds and furniture are washed daily with the same solution. Dirty laundry is put into sacks marked with red tape and all laundry and bed clothes are disinfected between each patient. Each patient has his own ointment tray, hand basin, bedpan and other utensils, where no provision is made for sterilization.

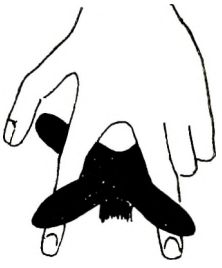

Fig. 3. The washing and disinfecting of hands.

PATIENTS

For hand washing and personal hygiene will use *solid soap*.

STAFF

Cleansing with disinfectants. For hand washing will use a *liquid disinfection* preparation (pHisoHex in dispensers).

Technique for disinfection washing with pHisoHex dispenser

1. Turn the tap with a two-finger grip, so as to dirty as little as possible (forefinger and middle finger astride the tap).
2. Wet both hands.
3. Hold one hand under the dispenser nozzle and press the pump button *once* with the other hand or forearm.
4. Rub the disinfectant liquid preparation well into both hands during at least 30 sec. (N.B. not under running water).
5. Rinse subsequently under running water.
6. Dry your hands, if needed (it is in fact better from the point of view of disinfection to let them dry naturally).

Handtowels. One towel at each washing place. Change towels several times a day. After a few hours the bacteria begin to multiply rapidly in a handtowel. This makes the towel itself increasingly infectious. *Dirty towels spread infection.*

Disinfection without cleaning. During rounds, dressings and bed linen changing, hands should be disinfected *between each patient.* This can be done by means of a spray bottle containing 0·1 % benzalkonium chloride solution.

Disinfection before operations. This is prescribed by the relevant head of clinic.

GOVERNOR.

1960. 1 *July*–31 *December*

Memorandum on cleaning bathrooms

After use the bathtub is scrubbed, thoroughly rinsed and washed out with a 0·1 % benzalkonium chloride solution. Each patient is supplied with a clean washing flannel. Neck bands and bath harnesses are rinsed or wiped off with a 0·1 % benzalkonium chloride solution between each patient. Bath brushes are only used when quite necessary and then they are steam-treated between each patient.

Disposable plastic gloves are recommended during all dressing operations and bathing of infectious patients.

1961. 1 *January*–30 *June*

A total of 1386 pHisoHex hand dispensers was installed throughout the hospital during the period March–June. As these were installed, all staff were instructed by the superintendents of departments concerning the use and significance of pHisoHex. The use by the staff of solid soap for hand washing was forbidden.

Scouring rags will be thoroughly cleaned in hot water and then placed in a 0·1 % benzalkonium chloride solution.

1961. 1 *July*–31 *December*

Memorandum on hand disinfection (see Fig. 3)

There will be only one hand towel at each wash place. This will be changed at least 4–6 times daily, and where necessary, even more often.

Memorandum on the disinfection of books

Books which have been borrowed by infectious patients will be disinfected in hot air for 3 hr. at 90° C., with the covers at an angle of 90° and the pages fanned out between.

Other instructions.

Bedpans, washing bowls, sputum mugs, urine bottles and similar articles will be sterilized or boiled each time after use. If this is not possible, then after careful mechanical washing they will be disinfected with a 0·1 % solution of benzalkonium chloride and then dried in air.

Lavatory seats will be wiped off several times a day with a 0·1 % solution of benzalkonium chloride.

Disposable plastic plates, mugs and glasses may be used for certain infectious patients.

ANTISEPTIC MEASURES

As can be seen from the aforementioned instructions, benzalkonium chloride has been selected as the general disinfectant for surfaces and articles of different types. The choice can be discussed from various viewpoints (Kjellander, 1960), but it was preceded by several laboratory and practical examinations (Juhlin & Ericson, 1960; Juhlin, Ericson & Willard, to be published) of the effects of different disinfectants (invert soaps, iodophores, Chloramine and Warexin).

It was possible to reduce the cost of this dinsinfectant considerably by distributing the original 50 % solution in bottles, with appropriate dilution marks, to all the wards, each of which subsequently did its own diluting. During 1962 the total cost of benzalkonium chloride disinfectant was £2000. The use of a phenol-soap-spirit solution, a 5 % chloramine solution or another invert soap solution, would have been three to ten times as expensive. The risk of skin injuries among the staff with this concentration of benzalkonium chloride is considerably less than with the prolonged use of several other substances (Medrek & Litsky, 1957). In special situations, however, when cleansing after a patient with open tuberculosis,

for example, a phenol-soap-spirit solution is used.* For daily floor washing only water is used, in order not to inactivate the invert soap.

The new laundry trolleys are a great hygienic and labour-saving improvement. The previous sack holder was difficult to handle and meant that the bed linen was usually thrown on to the floor, often together with used dressings, and then carried through the entire ward to where the sack holder was standing. These new trolleys can be pushed easily from bed to bed, besides which they are easily stored since they can be stacked one on top of the other (see Plate 1).

The previous formalin disinfection system for the laundry was replaced by a 7 hr. steam treatment at 90° C. Repeated tests had shown that this method completely destroys vegetative bacteria and often spores too. This method also saved a lot of time and space since there was no need for the unpacking of sacks for airing and subsequent re-packing.

The directives on the isolation of infectious patients could only be followed to a limited extent, since there was not much special isolation space in most of the departments. In certain situations it was possible to assemble several patients with the same type of staphylococcal infection in a small ward. The other directives concerning the handling of patients, however, were carried out in full.

One of the most difficult problems to solve was that of the complete cleansing and disinfecting of the hands of staff. Several of the published investigations deal only with pre-operative hand washing with different substances, and even though these publications give some guidance, none of them can be used as a background for the selection of cleansing and disinfecting media for the general hand washing of all types of staff. (Hopper, Beck & Wood, 1953; Göpel, Rücker & Schütz, 1958; Smylie, Webster & Bruce, 1959; Hurst, Stuttard & Woodroffe, 1960; Kjellander & Nygren, 1960; Lowbury, Lilly & Bull, 1960; Halvorsen & Hofstad, 1962).

On account of this, extensive comparative investigations on the disinfective properties of different preparations were carried out (soap, soap and rinsing in a 72% ethyl alcohol solution, 0·1% benzalkonium chloride, soap and thorough rinsing in water followed by disinfection with 0·1% benzalkonium chloride, liquid soap with 0·5% hexachlorophene, Nicasept and pHisoHex).

Ordinary soap and liquid soap with 0·5% hexachlorophene were entirely unsatisfactory. Nicasept resulted in rapid skin irritations and the soap/spirit combination was expensive and difficult to introduce. Even though it was possible in certain cases to achieve a satisfactory result with soap as a cleanser followed by benzalkonium chloride as a disinfectant, the effects were nevertheless often unsatisfactory. Both investigations gave equally satisfactory results with pHisoHex (Juhlin, Ericson & Willard, to be published).

Since ordinary bar soap can undoubtedly constitute a dangerous source of infection, (Kjellander & Nygren, 1959) and in view of the results achieved in this study, it was decided to adopt pHisoHex throughout the entire hospital as a cleansing and disinfecting medium for staff hand washing. pHisoHex fulfills practically all the requirements; good cleansing effect, 40% better than most

* Orthophenylphenol sodium, 500 g., 96% ethyl alcohol, 300 ml., 50% soap solution, 350 ml. This stock solution is diluted 1/20 before use.

soaps (Walter, 1952), good antiseptic effect established both before and during the investigations in question, persistence of action and minimum risk of allergy shown in various instances (Nelson & Stoesser, 1953; Hill, Butler & Laver, 1959), little or no risk of the fluid from the dispensers serving as a source of infection.

The only disadvantage to pHisoHex was the expense. Annual consumption cost is reckoned at £2414 as opposed to £690–£1034 for liquid soap.

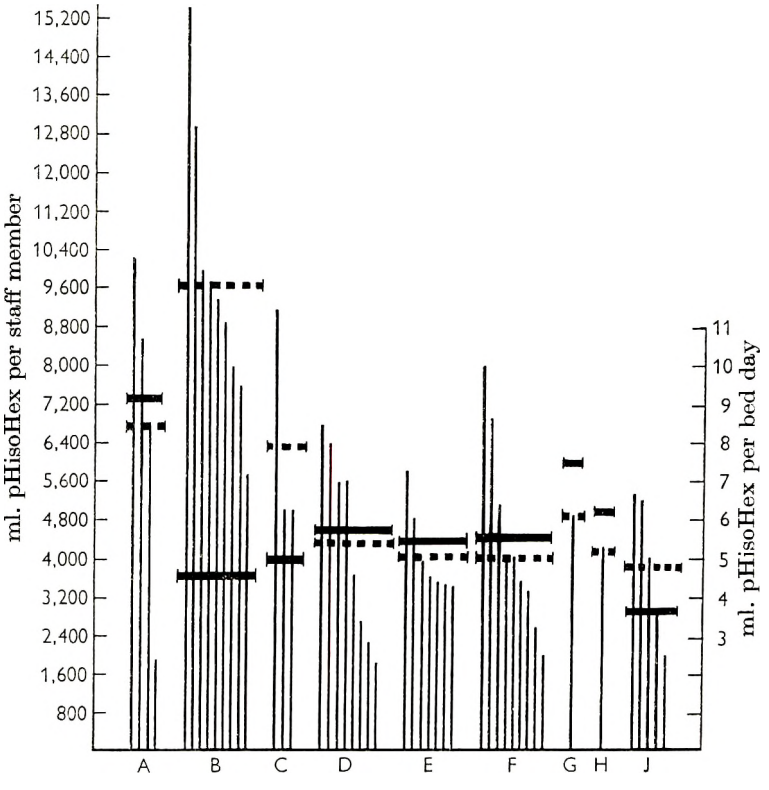

Fig. 4. Use of pHisoHex during 1962 in nine departments: A, infectious diseases; B, long-term diseases; C, internal chest diseases; D, internal medicine; E, obstetrics and gynaecology; F, general surgery; G, chest surgery; H, plastic surgery; J, orthopaedics. The columns show the use in ml. per staff member in each ward. ■■■. Mean use in ml. per staff member for each department; ▬▬ mean use in ml. per bed day for each department.

Fig. 4 shows the consumption during 1962. The individual columns show the consumption of pHisoHex, in ml. per staff member, for each ward in the various departments. Consumption varies considerably both between the different departments and between the different wards in each department. In several cases this may be explained by the fact that the older wards have fewer wash places, which naturally results in a reduction in washing frequency (ward A: 29 dispensers for 28 beds, ward B: 15 dispensers for 36 beds). In other cases the nature of the patients, material, type of treatment, etc. play an important role, for example: in the Department of Long-Term Diseases a fairly small staff looks after a large number of patients. Here the character of the treatment provides more frequent

contact between each individual patient and staff member, which results in a higher washing frequency. In other departments, or special wards with a large number of specially trained personnel for different duties, contact between staff and patients is not so frequent, resulting in a lower pHisoHex consumption per staff member, as in Plastic Surgery. The heavy dotted lines at right angles to the columns show mean consumption in ml. per staff member in each department. The

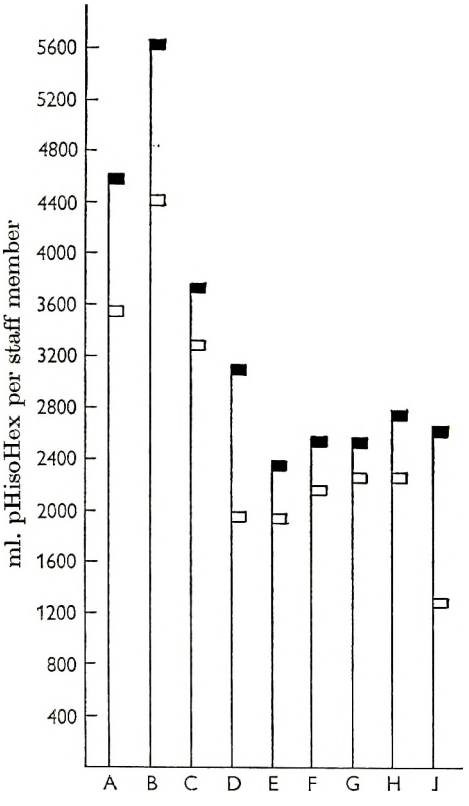

Fig. 5. Use of pHisoHex during the periods 1 October 1961 to 1 April 1962 and 1 October 1962 to 1 April 1963 at the nine departments. A–J, see Fig. 4. □, Ml. per staff member during the first period; ■, second period.

Departments of Infectious Diseases, Long-Term Diseases and Internal Chest Diseases have a much higher consumption per staff member than the others, all of which have about the same level of consumption.

If, on the other hand, we look at the mean consumption per bed day (shown on the table by the heavy unbroken lines) it will be seen that the Departments of Infectious Diseases and the Chest Surgery show the highest consumption, while the Departments of Long-Term Diseases and Internal Chest Diseases show the same or a somewhat lower consumption than the other departments. This latter division of consumption in relation to the number of bed days probably gives a more accurate picture of the hand disinfection requirements in relation to the number of patient contacts. Reckoned in ml. per bed day, the consumption of the

Department of Orthopaedics is much lower than any of the others. No particular explanation can be given for this.

On account of holidays and annual major cleaning operations, ward work varies in intensity during different parts of the year. As a rule, from 1 October to 1 April there is no interruption in the ward work procedure, and because of this the consumption of pHisoHex in this period was checked during two successive years. Fig. 5 shows only the mean consumption in ml. per department and per staff member (for technical reasons it was impossible to reckon consumption per bed day).

Common to all departments is a rise in consumption from the first half year period to the next. This rise is the result of a more steady consumption in departments of different types and also of an absolute increase in several of the departments. The Department of Orthopaedics has shown the largest percentage increase; in this case consumption was doubled.

The figures for consumption reported so far apply only to the wards. Total consumption for the entire hospital for 1962 amounted to 941 cans (4000 l.) of pHisoHex. This was used by a total of 2017 staff members (doctors, nurses, domestics, doctors' secretaries, physiotherapists, vocational therapists, midwives, junior nurses, student nurses, laboratory assistants and ward cleaners) which gives a mean consumption of 1866 ml. per staff member during 1962, compared with 5482 ml. per staff member in the nine departments investigated. Thus the total cost of pHisoHex during 1962 was £3212 (46,579 Sw. crowns); for the same year the number of bed days was 508,325, which gives a mean consumption of 7·4 ml. per bed day at a cost of 1·51 pence (0·09 Sw. crowns), compared with 5·4 ml. per bed day for the nine departments investigated. The latter figure does not include consumption in operating theatres, general consulting and service departments.

DISCUSSION

When evaluating the effects of the hygiene measures which have been carried out, one is faced with considerable difficulties. There are many extraneous factors which may affect the results; for example the presence of one or several actively infectious persons in a department, the creation of special wards within a department and the introduction of new, more effective, antibiotics.

In the investigations in question it has been possible to influence the effects of certain of these factors. Several years before the investigations were begun, the nose and throat flora of all staff in certain departments and wards were examined fairly regularly, and in certain cases treated. In 1958 it was made compulsory for all personnel with manifest staphylocccal infection to report it. These individuals were put on the sick list and given treatment, though they did not thereby suffer financial loss. The latter provision was made in order to encourage as many infected people as possible to report. Departments where the internal organization has meanwhile been more or less radically changed have not been included in the data. There have been no changes in the principles of examination, such as might result in a reduction in the number of tests. On the contrary, as the hygienic

work intensified, increasingly great care has been devoted to taking tests in every form of infectious state, even those of a rather prosaic character.

Detailed investigations carried out in surgical and infants' wards on the effects of the introduction of single hygienic measures have been published (Knörr & Wallner, 1957; Ravenholt, Wright & Multhern, 1957; Gillespie, Simpson & Tozer, 1958; Felton, Willard & Bass, 1959; Frappier-Davignon, Frappier & St-Pierre, 1959; Myers, Nimeck & MacKenzie, 1959; Plueckhahn, 1961; Caplan, 1962). Naturally, the scope of such investigations must be limited. The investigations reported in this paper have rather been based upon completely routine infections from eight different departments. These departments contained 1079 beds (331,340 bed days), and all belonged to a University Hospital with a total of 1872 beds (508,325 bed days). At this hospital a series of hygienic measures has rapidly been introduced and the frequency of infection investigated. It might therefore be possible for this type of follow up to be used to reflect more realistically the results which may be derived from different measures in practice.

The hygienic measures introduced in 1959 and 1960 were intended mainly as an attempt to reduce the sources of bacteria by tightening up the cleaning and disinfecting regulations for floors, furniture, bed clothes, etc., by isolating personnel with clinical staphylococcal infection, by treating the carriers with nose ointment and in certain cases with antibiotics and, as far as possible, by isolating '80/81' patients. Despite these measures, however, the frequency of infection continued to rise in 1959 and 1960 (for the two 6-month periods of 1960 see Ericson & Juhlin, 1965, fig. 1). The increase of the 'multiple-resistant' strains was particularly disturbing since the difficulty of treating patients effectively and the high frequency of '80/81' infections was a constant threat to the staff, who in many cases had to report sick with furuncles and similar complaints. In both figs. 1 and 2 of the previous work (Ericson & Juhlin, 1965) one notices in 1961, meanwhile, a distinct decline in the frequency of infection in all nine of the departments which were investigated. In certain of the departments this decline began during the first 6 months and at others during the second 6 months. The only measure which can be temporally associated with this decline in the frequency of infection is the introduction of pHisoHex as a hand disinfectant for all ward staff. This took place in April–June 1961. Many earlier investigations have shown the importance of thorough pre-operative hand disinfection for operating-theatre staff and have also reported the frequency of post-operative infections and their origins. Here, however, the pre-operative hand-washing procedure, the usual combination of soap-spirit, was not altered, so that in this case this factor cannot affect the result. Furthermore, the Department of General Surgery shows a very low frequency of infection: 1·41% in 1960 and 1·11% in 1962, compared, for example, with the Department of Long-Term Diseases: 15·78% in 1960 and 3·8% in 1962. These results demonstrate the vital need for a perfect hand disinfectant for specialist ward staff.

The majority of handbooks emphasize the importance of good hand hygiene in all hospital treatment work, (Williams, Blowers, Garrod & Shooter, 1960; Williams & Shooter, 1963) and in the investigation dealt with in this paper no reduction in

the frequency of infection was achieved until improved hand hygiene for staff members had been introduced. The vital significance of optimal hand disinfection in the struggle against nosocomial infections is illustrated by the following comparison. From October 1959 through the whole of 1960 dispensers containing 0·1 % benzalkonium chloride were used for hand disinfection, after washing with soap, in the Department of Long-Term Diseases; nevertheless the frequency of infection was not affected in this department until soap-benzalkonium chloride was replaced by pHisoHex (see Fig. 1). The number of dispensers was the same for each antiseptic.

The pronounced decline in the frequency of infection during 1961 can hardly have been the result of unknown or temporary circumstances affecting all eight of the departments at the same time. This decline must have been the result of improved hygienic measures. Even though there is good reason to believe that pHisoHex was the measure largely responsible for the decrease in the frequency of infections, it cannot be said that the other measures were of no consequence at all. It is quite possible that the final measure, i.e. pHisoHex, was introduced at just that time when the preceding measures had combined to produce a situation in which the introduction of pHisoHex could cause a decline in the frequency of infection.

The decline in the frequency of infection continued in most of the departments during 1962 also. Of particular significance was the pronounced reduction in '80/81' infections (a reduction of 66·4 %) and in infections with multiple-resistant strains (a reduction of 33·3 %) in the complete material from all eight departments (Ericson & Juhlin, 1965, Table 3). In certain of the departments the decline was even more pronounced, for example, in the Departments of Internal Chest Diseases and Obstetrics and Gynaecology, where there was a 70 % reduction in infections with '80/81' and 'multiple-resistant' strains. As mentioned previously, in the Department of General Surgery the frequency of infection was comparatively low, only 1·41–1·11 %, but despite this the number of infections here was reduced by a total of 31 % (Ericson & Juhlin, 1965, Table 2).

Similarly, the number of staff members who had to report sick or stay away on account of staphylococcal infections decreased from fifty-four in 1960 to thirteen in 1962.

Approximately 2000 hospital staff members have now used pHisoHex for over two years. A questionnaire distributed by the superintendents of the clinics to all staff members revealed that fourteen people suffered skin irritation, mainly dryness, through the use of pHisoHex and twenty-six people through the use of benzalkonium chloride. Thirty-two of these forty individuals were subjected to careful examinations in the Department of Dermatology. Epicutaneous tests were carried out with undiluted pHisoHex, 1 % hexachlorophene in vaselin, and 0·1 % benzalkonium chloride in aq. dest. on all thirty-two individuals. None of them showed any reaction to the pHisoHex or the hexachlorophene after 48 or 72 hr., while two reacted to the benzalkonium chloride. These two were able to use pHisoHex without discomfort. No less than fifteen out of the thirty-two were found to have suffered from eczema before beginning to use pHisoHex or benzal-

konium chloride, while six had also experienced difficulties with other kinds of washing agents or soap. During the investigations, objective lesions of a minor nature were visible in seven of the individuals while five of them suffered eczematous lesions. There was no evidence of allergy to pHisoHex during the course of these investigations.

The cost of these hygienic measures is more than compensated by the reduction in complicated infections. Indirectly this can save a large number of bed days and also decrease the number of days of sickness and absence from work for staff members.

SUMMARY

A hygienic programme, which has been developed and maintained during several years at a large Swedish university hospital, is presented in detail. The composition and working methods of the Infection Control Committee are described and some economic calculations given. The possible connexion between the hygienic measures and a marked decrease of the incidence of infections is discussed. This decrease did not occur until a satisfactory method for the routine hand disinfection had been introduced, using a commercial antibacterial cleaner containing 3% hexachlorophene.

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EXPLANATION OF PLATE I

Stackable trolleys, showing sack for dirty laundry and bucket with plastic bag interior for used dressings.

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Clinical and subclinical variola minor in a ward outbreak

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INTRODUCTION

Missing links in the chain of variola transmission are usually ascribed to unrecognized instances of clinically manifest infection or, less frequently, to instances of subclinical infection. Published virological studies of variola epidemics do not include a systematic search for subclinical infections, and in only two reports are isolated instances recorded. Verlinde & van Tongeren (1952) reported isolation of variola virus from the throat of a healthy contact of a patient with overt variola, a finding questioned by Dixon (1962) because of the long interval after contact. Rodrigues-da-Silva, Rabello & Angulo (1963) found significant complement-fixation titres in six asymptomatic contacts of housemates or schoolmates with overt variola minor. The present paper describes an outbreak of variola minor occurring in a hospital ward and, particularly, a serological survey aimed at disclosing subclinical variola.

MATERIALS AND METHODS

The locale and population

The outbreak occurred in the 4th General Medical Infirmary of the Santa Casa de Misericórdia, a charity hospital of the city of São Paulo, in 1959. The infirmary occupied the right wing of a floor, in one of the six buildings composing the hospital. All beds were located in a single, large ward and the infirmary also comprised a first-aid room, a dining room, a waiting room for visitors and two sets of water closet and bathroom (Fig. 1). Corridors and stairways connected the infirmary with the rest of the floor and with other floors from the building.

On admission of the introducer of infection, there were thirty-four patients, all adult males, in the ward. Two deaths, one discharge and five admissions occurred during the outbreak. The patient occupying bed twenty-one on admission of the introducer died on 29 July 1959, this bed being soon occupied by one of the admissions. These two patients will be referred to as 'patient 21A and 'patient 21B' respectively. The same nomenclature is applied to the patients successively occupying bed 33, the first of which, 'patient 33A', was discharged on 11 August, the second, 'patient 33B', being admitted on 12 August. The third admission occupied bed 23 which was empty on admission of the introducer. The fourth admission occupied bed 36 for a short interval (see below) while the fifth admission did not occupy a bed but slept on a mattress placed on the floor every night and removed the next morning. This mattress is represented as bed 0 in Fig. 1, the

patient being referred to correspondingly in the text. The patient from bed 17, who was suffering from Chagas disease with heart involvement, died on 2 August.

Three physicians and five male nurses took care of the patients and there was an orderly for the infirmary. While several patients were bedridden, others moved around through the infirmary and sporadically visited other infirmaries from the same or other buildings. Seven patients were suffering from allergic dermatitis, seven patients exhibited leishmaniasis, four patients had leg ulcers, three patients showed blastomycosis and other patients were suffering from liver cirrhosis, nephrosis, nephritis, Chagas disease, verminosis, etc. Only a male nurse had previously suffered from variola in the study population group.

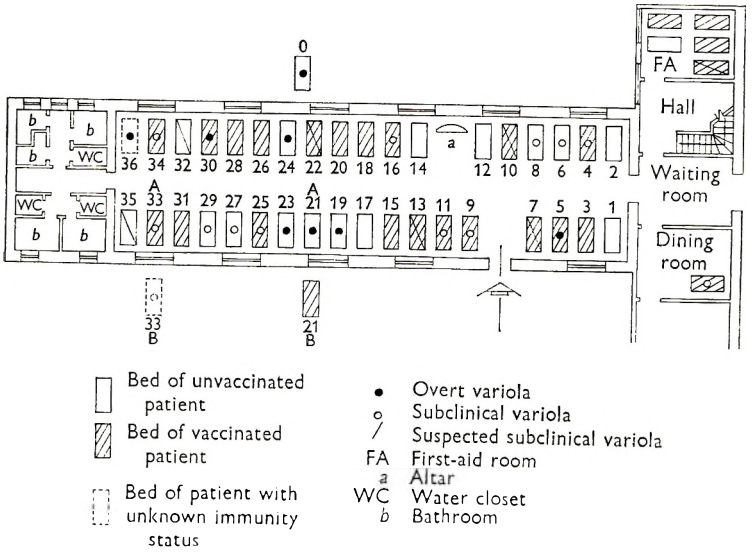

Fig. 1. Spatial relations among individual infections.

Method of investigating the outbreak

When the occurrence of variola was recognized (see below), a case-finding survey was conducted in retrospect and all inmates and staff members were examined and questioned about occurrence of skin or constitutional manifestations attributable to variolous rashes or *variola sine eruptione* during the interval after admission of the introducer. Demographic characteristics were also recorded and more clinical examinations and questioning were later made at variable intervals in all patients and staff members. Cases of overt variola were identified through the classical clinical criteria, length of interval between illness onset in the infecting and infected cases, antibody titrations and, in three cases, by virus isolation from skin pocks. The previous immunity status was determined through inspection of the usual vaccination sites and careful questioning. Lack of co-operation made it impossible, in a few instances, to obtain some data.

An environmental survey was made, aimed at disclosing the spatial relations among cases and contacts and, particularly, the personal associations which might have influenced spread of infection. Since the population of a ward offers peculiar

facilities for disclosing subclinical infections, a serological survey was also conducted. Patients and staff members were bled on 3, 12 and 28 August that is, 28, 37 and 53 days after introduction of infection in the ward. The earliest bleeding was made on the day preceding a mass vaccination of the ward population. Two patients were not bled and several donors were bled only once or twice because of death, discharge, admission during the outbreak or temporary absence. No blood could be obtained from the attending physicians because of their reluctance.

Blood obtained by venipuncture was kept overnight at 4° C.; the serum was then separated and stored at −25° C. Before testing, sera were inactivated at 56° C. for 30 min. Complement-fixation (CF) and haemagglutination-inhibition (HI) tests were conducted on each serum following the techniques of McCarthy & Downie (1953). The Instituto Butantan strain of vaccinia virus was employed in the preparation of antigens and hyperimmune rabbit serum. In some tests, serum from a donor with virologically confirmed, clinically typical generalized vaccinia was employed as a positive control, while, in other tests, hyperimmune rabbit serum was used. All sera from a given donor were simultaneously tested by one of the tests. The lowest serum dilution employed in the CF was 1/5, while in the HI it was 1/10. Titres are expressed in terms of serum dilution before addition of the CF antigen or the haemagglutinin.

From each of three patients with a presumptive variolous eruption, a specimen was collected for virus isolation. The specimen consisted of liquid content from several pocks aspirated with a capillary pipette as well as of pieces excised with forceps from the roof of these or other pocks. This material was placed in a tube containing 0·5 ml of broth and then stored at −25° C. until testing, when the solid material was ground and penicillin and streptomycin were added to the specimen for preventing bacterial growth. Embryonated hen eggs were inoculated on the chorioallantois by the usual technique. Besides serial passaging on the chorioallantois, the strains were inoculated on rabbit skin by scarification. One of the strains was subjected to a neutralization test according to McCarthy & Downie (1953) using serum from a rabbit hyperimmunized with the Instituto Butantan strain of vaccinia virus, as well as to the test devised by Helbert (1957) for differentiation between variola minor strains and variola major strains after previous titration on the chorioallantois. Each egg from a series of ten was inoculated with 10,000 pock-forming doses, while each egg from another series of ten was inoculated with 100,000 pock-forming doses and embryo mortality was determined at daily intervals until the 7th day after inoculation.

RESULTS

Course of the outbreak

On 6 July 1959, an 18-year-old man was admitted to the ward because of generalized leishmaniasis and occupied bed 24 (Fig. 1). He also had a pock eruption the identity of which was not suspected mainly because attention was focused on mutilating lesions of leishmaniasis frankly predominating in the clinical picture. The pock eruption started 5 days before admission, after a pre-

eruptive illness lasting 2 days. The patient had never been vaccinated against variola and had not suffered from this disease. Suspicion of variola arose 23 days after his admission, when a pathologist found umbilicated pustules on the face and extremities of patient 21 A who had just died. By the time variola virus was isolated and identified in the material collected from the cadaver pustules, most

Table 1. *Characteristics of cases of overt variola*

Bed no.	Age	Immunity status	Laboratory findings	Date of illness onset (1959)	Behaviour during the outbreak
24	18	No scar	High CF titre. Rather high HI titre	29 June	Introducer of infection
30	51	37-year-old scar	Very high CF titre	18 July	Secondary case (first generation)
19	25	No scar	Fairly high HI titre	18 July	Secondary case (first generation)
5	15	10-year-old scar	Very high CF titre. High HI titre	19 July	Secondary case (first generation)
21-A	68	No scar	Virus isolation	20 July	Secondary case (first generation)
23	28	No scar	Virus isolation. Eightfold HI titre increase	1 August	Secondary case (second generation)
36	?	?	—	2 August	Secondary case (second generation)
0	?	No scar	Virus isolation. Fourfold HI titre increase	22 August	Secondary case (third generation)

Scar means vaccination scar; no patient had previously suffered from variola.
Admission of the introducer was made on 6 July 1959, when he was in the 8th day of illness.
The date of illness onset in the patient from bed 36 is approximate.
? the pertinent information is missing.

pocks on the introducer (bed 24) were in the crust stage. Six secondary cases had already appeared and these and the introducer were soon identified as cases of variola. The introducer had been moving around through the infirmary and had even gone to another building to be photographed.

Visits of friends and relatives were forbidden and a mass vaccination of the ward population was conducted on 4 August. The patients with generalized allergic dermatitis were spared and twenty-three patients and one staff member were vaccinated, but this was only successful in the patients from beds 7, 11 and 12. No current or terminal disinfection of the ward was carried out nor were patients with variola placed in an isolation room.

A case occurred in a Japanese who did not belong to the infirmary but who was allowed to sleep on bed 36 for two nights on about 19 and 20 July, returning to his

original infirmary to leave for an unknown address soon after the appearance of the variolous eruption. The appearance of this eruption was reported to the authors by the staff of the other infirmary, in which no other case appeared. A total of eight cases of overt variola was recorded and some characteristics of these cases appear in Table 1. The seven cases secondary to that of the introducer were seemingly grouped in three generations and the last case started on 22 August. No case of overt variola occurred among staff members or visitors nor in the adjoining infirmary or elsewhere in the hospital.

Clinical findings were typical of variola minor and did not include any unusual characteristics. All cases showed a benign eruption consisting of discrete pocks, after intense systemic manifestations lasting the usual time. The only fatal outcome could not reasonably be attributed to variola, since the eruption in this case did not include haemorrhages or other manifestations of malignant character and was not even confluent. Death might be attributed to pneumonia complicating the generalized cysticercosis with right hemiplegia from which this 68-year-old patient 21 A was suffering. No post-mortem examination could be made because of refusal of relatives.

Identification of the outbreak

All three specimens of pock material yielded variola virus. The virus was identified by: (*a*) the macroscopic morphology of chorioallantoic lesions on isolation and the maintenance of this morphology in three serial passages; (*b*) histological examination of inoculated membranes; (*c*) frequent embryo survival and absence of membrane haemorrhages; (*d*) absence of definite lesions on rabbit skin inoculated through scarification; (*e*) significant neutralization (more than 50 %) of the virus from the patient occupying bed 23 by antiserum prepared in the rabbit; (*f*) Results typical of variola minor strains in Helbert's test conducted on the same strain.

All cases of clinically manifest infection were identified by typical clinical and epidemiological findings and, particularly, by aetiological (laboratory) data from all but one case (Table 1). The pock eruption in the introducer (patient from bed 24) was identified by: (*a*) A 1/160 CF titre and a 1/160 HI titre in the absence of previous variola or successful vaccination; (*b*) a fourfold decrease of the CF titre and a twofold decrease of the HI titre within 9 days after the first bleeding; (*c*) a typical pre-eruptive illness followed by an eruption of morphology, distribution and course typical of variola minor; (*d*) occurrence of four laboratory-confirmed cases of variolous eruption within 2 days and all appearing 12–14 days after the admission of the introducer (Table 1).

One case from each of the three generations of secondary cases was identified by virus isolation from pocks (Table 1). In the patients from beds 23 and 0 a significant (fourfold or higher) titre increase of the HI antibody was also found. A secondary case occurring in the patient from bed 19 was identified through a 1/320 HI titre falling to 1/40 within 9 days, in the absence of previous variola and successful vaccination. The two secondary cases occurring in the patients from beds 5 and 30 had very high CF titres of 1/640 and 1/320 falling within

25 days to 1/160 and < 1/5 respectively. This rapid fall adds to the significance of the early titres. Only the case occurring in the patient from bed 36 was not identified through virological data for reasons already mentioned.

Table 2. *Serological evidence of subclinical variola*

Patient's bed no.	Complement fixation	Haemagglutination inhibition	Immunity status
8	< 1/5	< 1/10	No scar
	< 1/5	< 1/10	
	< 1/5	1/20	
33-B	N.D.	N.D.	Unrecorded
	< 1/5	< 1/10	
	< 1/5	1/160	
Ward orderly	1/40	1/160	6-year-old scar
	1/160	1/640	
	1/20	1/160	
6	1/40	1/160	No scar
	1/40	1/160	
	1/20	1/80	
29	1/10	< 1/10	No scar
	1/10	< 1/10	
	1/5	< 1/10	
34	1/320	1/160	32-year-old scar
	1/640	1/80	
	N.D.	N.D.	
16	1/320	1/320	39-year-old scar
	1/160	1/640	
	1/40	1/640	
4	1/160	1/640	30-year-old scar
	1/80	1/640	
	N.D.	N.D.	
11	1/80	N.D.	20-year-old scar
	1/40	1/20	
	1/20	1/20	
25	1/40	1/80	26-year-old scar
	1/40	1/80	
	N.D.	N.D.	
9	1/80	1/20	15-year-old scar
	1/80	1/10	
	N.D.	N.D.	
27	< 1/5	1/160	No scar
	< 1/5	1/80	
	N.D.	N.D.	
33-A	< 1/5	1/320	35-year-old scar
	N.D.	N.D.	
	N.D.	N.D.	

Patient 33A was bled only on 3 August, while patient 33B was bled on 12 and 28 August. Each of the patients from beds 4, 9, 25, 27 and 34 were bled only on 3 and 12 August. The remaining patients were bled on 3, 12 and 28 August.

N.D. Not done because no serum was available.

Scar. Vaccination scar. No patient had previously suffered from variola.

Subclinical variola

Table 2 presents serological evidence of current infection in donors without clinical manifestations of variola according to the following criteria: (*a*) a fourfold or greater increase of either CF or HI titre; (*b*) a 1/10 or higher CF titre in a donor without previous variola whose last successful vaccination occurred 15 or more years before or had not occurred at all; and, (*c*) a fairly high (1/160 or higher) HI titre in a donor without previous variola and whose last successful vaccination had occurred 35 years before or had not occurred at all. In some published reports, titres are expressed in terms of the final serum dilution, after addition of the antigen, and this makes them higher than the corresponding titres presented here. It should be emphasized that, in this outbreak, the first and second mass bleedings were made after onset of six of the seven secondary cases while the third bleeding was made 6 days after onset of the last case.

A fourfold or greater rise of titre was observed in two patients and one staff member (Table 2). The patient from bed 8 had no vaccination scar or previous variola and showed a fourfold increase of the HI titre. Patient 33B, whose immunity status was not found in the records, was admitted on 12 August and bled for the first time on the same day. This serum was negative in both CF and HI tests, while the serum collected on 28 August exhibited a 1/160 HI titre and negative CF titre. The ward orderly, who took care of patients' meals and clothing, showed a four-fold increase of both CF and HI titres. These titres fell rapidly during the next 16 days. This woman had been successfully vaccinated for the first and only time 6 years before the outbreak. Moreover, the CF was negative in all other (five) staff members bled and the same applies to the HI, except for a 1/80 titre found in the only serum obtained from a male nurse.

Eight patients did not show a rise of titre but showed significant (1/10 or higher) CF titres (Table 2). The patient from bed 29 twice showed a 1/10 CF titre which declined to 1/5 in the third serum, while the patient from bed 6 showed higher CF and HI titres in his three sera and these also declined in the third serum. These two patients had had no previous variola or successful vaccination. The remaining six patients had not suffered from variola but had successful vaccination 15–39 years before and this would not justify the CF titres observed. In each of these six patients, the CF titre was significant in all sera and most titres were high and usually declined in the last serum, a finding also made in patients with overt variola. Moreover significant, at times high, HI titres supported the CF titres observed in these patients.

The patient from bed 27 had not suffered from variola or successful vaccination and showed a rather high HI titre in the first serum, with a lower titre in the second serum (Table 2). Patient 33A showed a fairly high HI titre which cannot be ascribed to a previous successful vaccination occurring 35 years before, especially since negative or low HI titres were found in several patients with more recent successful vaccination.

Inconclusive serological evidence of subclinical variola was found in some persons not included in Table 2. The patients from beds 10 and 32 both showed HI titres

of < 1/10 on 3 August and 12 August and both showed a rise to 1/10 on 28 August. The two negative results form a control for the titratable but low titre of the third serum from each patient, but no four-fold increase was observed in either of these two patients, one of whom (patient from bed 32) had had no previous variola or successful vaccination. The patient from bed 13 showed a fairly high (1/320) HI titre on 3 August which fell to 1/80 by 12 August. This rapid decline within 9 days and the fairly high titre in the early serum strongly suggest that current infection had occurred in this patient who had been revaccinated 8 years before. The patient from bed 22, who occupied a bed adjacent to that of the introducer of infection, and who had not suffered from variola, showed a 1/80 HI titre in all three sera. This finding can hardly be attributed to previous successful vaccination 16 years earlier. The patient from bed 35 had no vaccination scar nor previous variola and showed a 1/5 CF titre in the early serum. This is significant according to Downie & McCarthy (1958), although it is not so according to the last criterion of Downie (1959). The 1/5 CF titre became negative in the serum collected 9 days later from this patient, and the HI titre also declined from 1/20 in the first serum to 1/10 in the second serum. A male nurse showed a 1/80 HI titre in the only serum obtained from him and he had had variola and successful vaccination more than 20 years before, while three other male nurses successfully vaccinated 6, 6 and 7 years before, respectively, showed negative HI and CF titres in all their sera.

In addition to this inconclusive evidence of subclinical variola, there was a significant increase of the CF titre in the sera from the patient occupying bed 7 and this increase might perhaps be attributed to subclinical variola instead of to a successful vaccination on the day (4 August) following the first bleeding because: (*a*) this patient occupied a bed adjacent to bed 5, of a patient with overt variola in the first generation of secondary cases; (*b*) vaccination did not provoke CF antibody response in the remaining two patients successfully vaccinated on 4 August; (*c*) vaccination does not provoke CF antibody response (Sindo & Nisimura, 1940; Herrlich, Mayr & Munz, 1956); and, (*d*) significant titre increases were observed in the ward orderly (Table 2) who was not vaccinated during the outbreak nor showed clinical manifestations of variola.

DISCUSSION

The identity of the disease was clearly established by clinical and epidemiological findings and, particularly, by virus isolation or antibody titrations in seven of the eight persons with overt variola. Instances of subclinical variola were deduced from serological evidence according to three criteria, the first of which, a significant titre increase, is the classical criterion and has general acceptance, while the remaining criteria are based upon known peculiarities of the persistence of variolous and vaccinial CF and HI antibodies. With an unconfirmed exception, reports of CF antibody response either show the persistence of this antibody for only a few months after successful vaccination or variola (Andres *et al.* 1958; McCarthy, Downie & Bradley, 1958; Downie & McCarthy, 1958), or the non-

appearance of CF antibody after successful vaccination (Sindo & Nisimura, 1940; Herrlich, Mayr & Munz, 1956; McCarthy *et al.* 1958) or after variola minor (de Jongh, 1956; Herrlich, Mayr & Mahnel, 1959). The HI antibody does not always appear after successful vaccination (McCarthy *et al.* 1958) but, when present, it persists longer than the CF antibody, although after 1 year its titre is low or negative (Herrlich *et al.* 1956; McCarthy *et al.* 1958). The latter finding has also been made in patients who had overt variola (Downie & McCarthy, 1958; Herrlich, Mayr & Mahnel, 1959). Unpublished work in this laboratory disclosed negative CF in a rather large proportion of patients suffering from variola minor or successful vaccination, as well as a rapid and marked decline of the CF titre and a definite decline of the HI titre within a few months after variola minor, generalized vaccinia or successful vaccination.

It is remarkable that at least four cases of subclinical variola occurred in fully susceptible individuals, that is, in persons without previous variola or successful vaccination. Moreover, three of these patients had not even suffered from varicella, a disease which might be confused with variola minor, and one of these three showed a significant antibody titre increase. In addition, the fully susceptible patients from beds 32 and 35 showed very suggestive though inconclusive evidence of subclinical variola.

Regarding the finding of subclinical variola in this outbreak, it should be remembered that recent work has established the actual occurrence of subclinical infections by the viruses of mumps, equine encephalomyelites, Japanese encephalitis, poliomyelitis, Coxsackie, etc. Moreover, virological and epidemiological studies disclosed that subclinical infections are the explanation for the apparently low infectiousness of mumps in spite of infection by mumps virus being highly communicable, affecting most persons in a manner essentially similar to that of measles virus (Meyer, 1962).

SUMMARY

A variola minor outbreak in a 36-bed hospital-ward comprised seven cases of overt variola after the first case. Clinical and epidemiological findings were typical, seven of the eight cases of overt variola being confirmed by virus isolation or antibody titrations. In addition, thirteen definite and seven possible instances of subclinical variola were deduced from complement-fixation or haemagglutination-inhibition tests. A discussion is made of the validity of serological criteria of variolous and vaccinial infections.

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The mechanism and prevention of cross-infection in dermatological wards

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Much of the extensive work on hospital cross-infection has of necessity been opportunist associated with the occurrence of sporadic outbreaks of infection. Investigations concentrated on such outbreaks cannot give a true picture of the general problem, and the value of any counter-measures which may have been introduced is not easily assessed.

A pilot study, carried out in a large dermatology department, revealed an ideal model for the planned, long-term investigation of hospital infection (Selwyn, 1963). An endemic situation was uncovered in which the extensive skin lesions of in-patients were readily colonized by the abundant pathogenic bacteria present in the environment. Many of the infected patients became in their turn prolific dispersers of pathogens. A detailed study of these processes was facilitated by the long periods of hospital treatment which many patients with skin diseases require. Further work was therefore carried out in this neglected field to investigate the dynamic relationship which exists between the bacterial flora of these patients and that of their environment. The effect of simple anti-bacterial measures upon this relationship was also studied during two of the four stages of the investigation. The principal aim here was to assess the relative importance of nasal carriers and patients with infected lesions in the aetiology of hospital infection.

MATERIALS AND METHODS

Sampling procedures

The investigation was carried out over a 2-year period in three dermatological wards. These are housed in a relatively modern pavilion, within a large teaching hospital. The two main wards, situated on different floors, are 68 ft. long, 28 ft. wide and 12 ft. high; each contains sixteen beds. The third ward, containing eight beds, is 32 ft. long; the width and height are as in the main wards.

The degree of bacterial contamination was measured on two days of the week at representative sites in each ward, and in the adjacent day-room and bathroom. Air counts were performed with pairs of 3·5 in. Petri dishes containing blood agar and 7·5% salt–milk agar respectively. These were exposed for 2 hr. periods as 'settle plates' and during each period similar plates were exposed in a 'Casella' slit sampler (but this could not be operated in the bathrooms). Solid surfaces were examined by contact-transfer using self-adhesive cellulose tape in a similar manner to that used by Thomas (1961) for swabbing skin. Direct impressions

were made from bedding and other fabrics on solid media (Rubbo & Dixson, 1960). Sweepings of settled dust were examined by shaking 100 mg. of dust in 10 ml. of nutrient broth, and carrying out bacterial counts upon the supernatant by the method of Miles & Misra (1938). The fibre composition of the dust was also determined (Pressley, 1958).

From each patient on admission, and at weekly intervals, swabs (moistened in 3·8 % sodium citrate solution) were taken from the anterior nares and from a typical area of the skin lesion. Nasal swabs were plated on to milk agar, free from excess salt, while lesion swabs were plated on to blood agar and salt–milk agar. All swabs were finally incubated in 10 % salt broth. In addition, nasal swabs and hand impressions on milk agar plates were taken regularly from the ward staff.

From all primary cultures on solid media colonies thought to be *Staphylococcus aureus* were subcultured on to nutrient agar and tested for coagulase production. Representative strains were later tested for pigment production on 1 % glycerol monoacetate agar (Willis & Turner, 1962). Bacteriophage typing was carried out upon all coagulase-positive staphylococci, by the methods of Blair & Williams (1961). Phage typing of *Pseudomonas pyocyanea* was performed by the method of Gould & McLeod (1960). Disk-diffusion sensitivity tests were carried out on all the pathogenic bacteria isolated.

Anti-bacterial measures

After an initial period of observation (February to mid-October 1962), anti-bacterial procedures were applied during the second stage (mid-October 1962 to March 1963) in the two main wards. Twice-daily nasal disinfection was introduced in the female ward, using 'Soframycin' nebulizers (1·25 % framycetin and 0·005 % gramicidin). The two adjacent baths were disinfected and cleansed using 1 oz. of concentrated hypochlorite solution mixed with detergent in a gallon of hot water (Boycott, 1956). In the main male ward all skin lesions were treated with 'Rikospray Antibiotic' (neomycin, bacitracin and colistin sulphate), before dressings were applied. During stage III (October to late December 1963), lesions were sprayed in the female ward, while the male ward received no special attention. In the final stage (late December 1963 to May 1964), antibiotic spraying was entirely withheld. Carry-over of anti-bacterial substances was avoided by swabbing immediately before spraying procedures were performed. In addition, when no growth was obtained from both the liquid and solid media, minimal inocula of a sensitive strain of *Staph. aureus* were introduced into the salt broths. Growth occurred in all cases.

RESULTS

Environment

The air. The histograms in Figs. 1–3 show the trends in the air counts of *Staph. aureus*. The busy period between 9 a.m. and 1 p.m. is represented throughout. Total bacterial counts are not shown in the figures: although these counts were usually high, they did not show the marked fluctuations seen in the levels of

Staph. aureus. In the bathrooms *Staph. aureus* constituted over 40 % of the total count on several occasions. Generally, however, it constituted between 2 and 5 % of the total in the wards, bathrooms and day-rooms.

Consistently high counts of *Staph. aureus* were obtained in the small ward, and

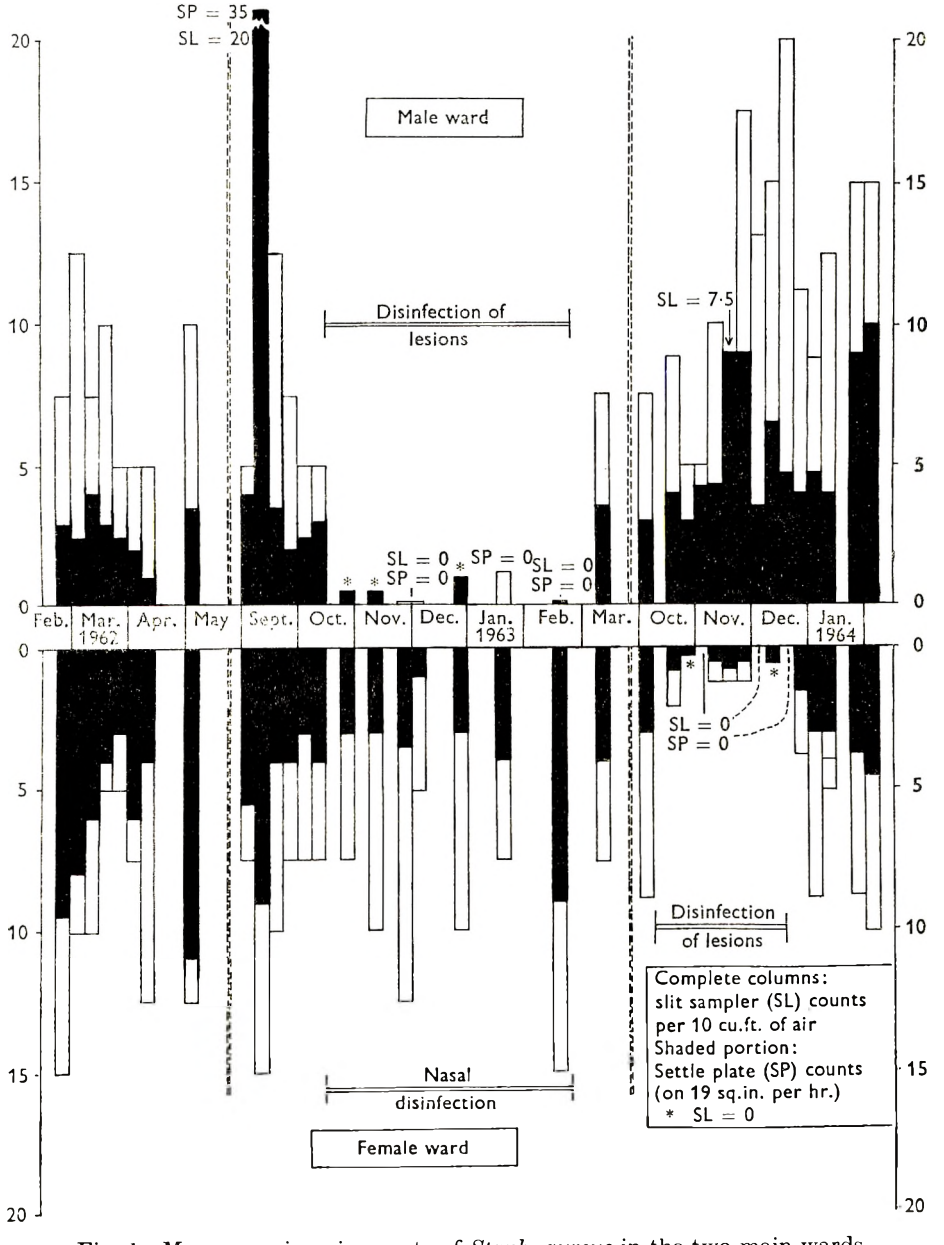

Fig. 1. Mean morning air counts of *Staph. aureus* in the two main wards.

in the male and female wards before mid-October 1962 (stage I), and after December 1963 (stage IV). This is in striking contrast to the levels seen in the male ward during stage II, when the skin lesions were being sprayed, and in the female

ward during the corresponding period (stage III). Nasal disinfection *per se* produced no significant change (female ward, stage II).

During the course of a day's sampling, large fluctuations were often seen in the counts of individual phage types, especially in the bathrooms. Apart from *Staph. aureus*, coliform bacilli, *Proteus mirabilis*, *Ps. pyocyanea* and non-haemolytic

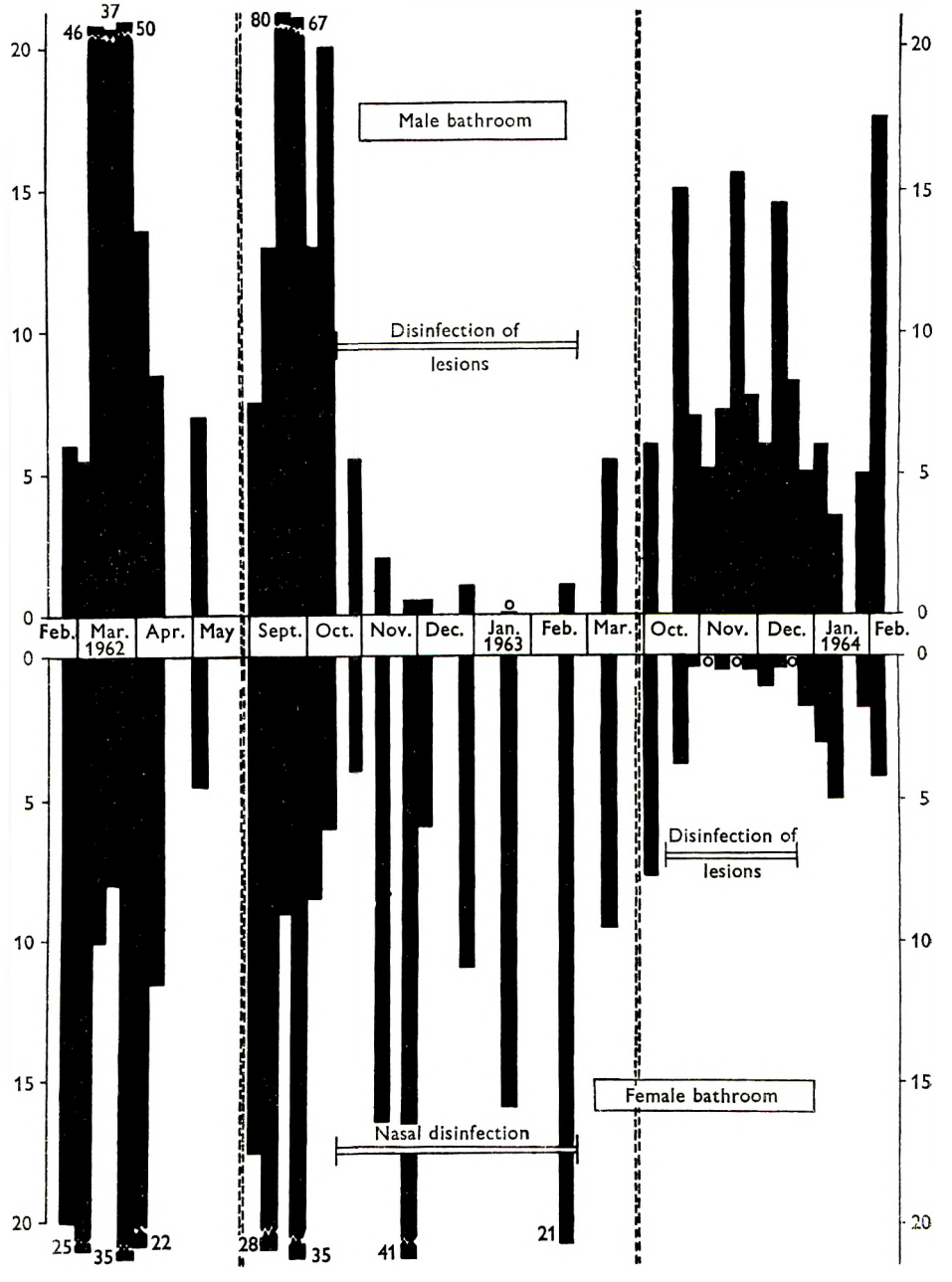

Fig. 2. Mean morning settle-plate counts of *Staph. aureus* in the main bathrooms. (No. of particles falling on 19 sq. in. per hr.)

streptococci were not infrequently obtained in air samples. The bathrooms again were the most frequent source of these miscellaneous organisms.

The diameters of air-borne particles carrying pathogens were calculated from Petri ratios (Lidwell, 1948), and were found to range widely from 4 μ to over 50 μ. This large scatter was confirmed by direct measurements using a size-grading impaction sampler, as described by Lidwell (1959), although this does not differentiate particles in the heaviest fraction (above about 18 μ in diameter).

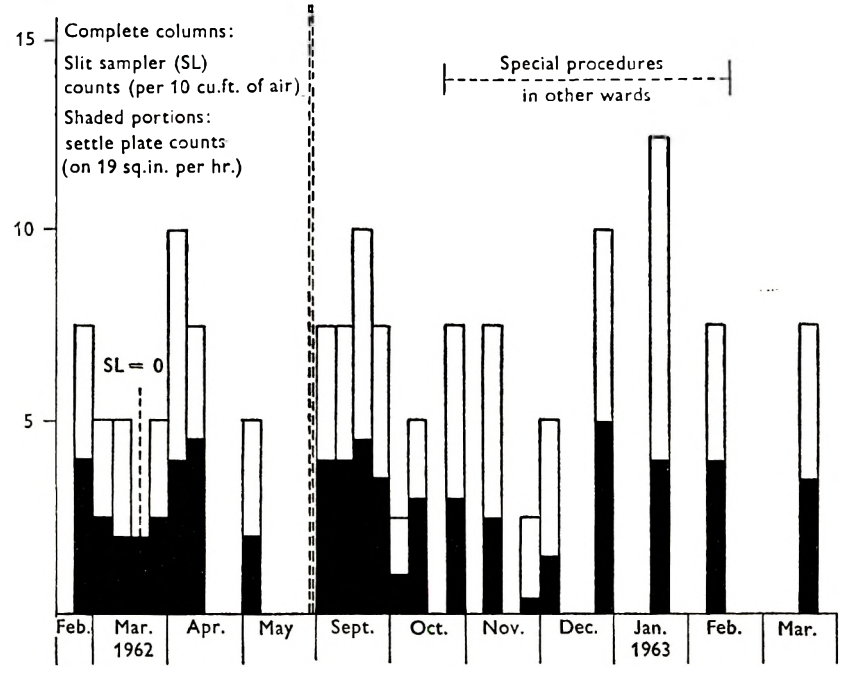

Fig. 3. Mean morning air counts of *Staph. aureus* in the small ward. (Detailed sampling was discontinued after March 1963 as the ward was no longer in normal use.)

The baths. The degree of bacterial contamination of the baths is shown in Table 1. After stage II, the staff in the female ward voluntarily continued to use Boycott's cleansing procedure—but not always adequately. The cleansing of the two male baths was very unsatisfactory throughout the study. Serial samples taken from the baths during the busy period gave clear evidence of a cumulative process of contamination, despite the customary addition of about 30 ml. of cetrimide solution to each patient's bath water. This seemed to exert no bactericidal effect, but might have favoured the Gram-negative bacilli in mixed bacterial populations.

The wards. Bedding, and especially the sheets, usually yielded heavy cultures of the prevalent pathogens—even within a few hours of a change. Almost one-third of the samples yielded strains other than those of the bed occupant. The bedding was, however, free of pathogenic bacteria before use.

Broth washings from many of the samples of settled floor-dust yielded remarkably heavy growths of Gram-negative bacilli, notably of *Ps. pyocyanea* and coliforms (up to 10^4 organisms per ml.); but *Staph. aureus* predominated in more

than half of all the samples. Cellulose constituted the great majority of fibres in these samples, though much of the dust was of an amorphous protein nature and produced a strong odour of burning keratin when ignited. Often this debris contained recognizable epidermal scales.

Heavy contamination was usually found, too, on objects as varied as door handles, taps, television sets and dressing trolleys.

Table 1. *Significant cultures of pathogens obtained from the communal baths*

Ward	Stage	No. of tests	Positive cultures*	
			Before cleansing	After cleansing
Male	I	42	—	37 (10)
	II	30	9 (5)	6 (4)
	III	44	36 (5)	22 (4)
	IV	30	25 (4)	14 (0)
Female	I	42	—	35 (8)
	II	30	21 (1)	2 (1)
	III	44	8 (1)	1 (0)
	IV	30	18 (5)	4 (0)

Note. Figures in parentheses refer to numbers of cultures with predominant Gram-negative bacilli.

— = Not done.

* Two or more colonies of *Staph. aureus* or pathogenic Gram-negative bacilli from a 2 sq.in. transfer strip.

Patients

Eczema and psoriasis were the two commonest diagnoses—113 and 114 cases respectively—and the incidence of acquired infection was similar in the two groups (43% and 35% respectively). The clinical significance of infection differed considerably, however, for the infected eczemas could often be diagnosed clinically, whereas most of the psoriasis lesions which yielded heavy cultures of pathogens showed no clinical evidence of infection. The highest incidence of infection with Gram-negative bacilli was found in the third most frequent diagnostic group—the varicose eczemas with ulceration. Of thirty-four such patients, twenty-one acquired infection in hospital, and in twelve of these Gram-negative bacilli predominated.

Tables 2 and 3 summarize the results obtained from lesion and nasal swabs. Only 'moderate' to 'heavy' primary cultures are recorded.

Over the 2-year period the incidence of significant infection in the skin lesions at the time of admission varied between 35% and 60%. Approximately 50% of the patients, however, acquired significant infection whilst in hospital during stages I, III and IV in the male ward, stages I, II and IV in the female ward, and stages I and II in the small ward. Sixty-five of the 129 hospital-acquired infections were due to cross-infection in previously 'clean' lesions; Gram-negative bacilli were responsible for 16% of these. Three-quarters of the remaining acquired infections took the form of super-infections, and 29% were due to Gram-negative bacilli.

Sixteen of the infections acquired in hospital were caused by strains of *Staph. aureus* present in the anterior nares on admission ('autogenous' in Table 2).

Nasal disinfection (female ward, stage II) produced no obvious change in the incidence of cross-infection, although no autogenous infections were seen. In contrast, during stage II (male ward) and stage III (female ward), only two instances of transient, acquired infection were seen. One appeared to be autogenous in a heavy carrier; the other was due to *Pr. mirabilis*.

Table 2. *Pre-existing and hospital-acquired infection in skin lesions*

			No. with infection			
				Acquired in hospital		
Ward	Stage	No. of patients	Upon admission	Primary cross-infection	Super-infection	Auto-genous
Male	I	46	20 (1)	11 (1)	8 (2)	4
	II	37	14	1 (1)	0	0
	III	30	11 (1)	12 (1)	4	1
	IV	37	18*	6	5 (1)	3
Female	I	45	21 (3)	7 (3)	11 (2)	3
	II	44	21 (6)	8 (2)	10 (6)	0
	III	28	13 (4)	0	0	1
	IV	40	17 (3)	12 (1)	3 (2)	2
Small	I	17	6 (1)	4 (1)	2	2
	II	18	11 (2)	4	5 (1)	0

Note. Figures in parentheses refer to numbers of cultures with predominant Gram-negative bacilli.

* Including one case of eczema infected with group A streptococci (*Staph. aureus* predominated in the remaining infections).

Table 3. *Nasal carriage of* Staph. aureus *in patients*

			Nasal carriage		
				Acquired in hospital	
Ward	Stage	No. of patients	Upon admission	Primary infection	Super-infection
Male	I	46	27	5	3
	II	37	17	1	0
	III	30	9	6	0
	IV	37	15	6	3
Female	I	45	26	6	3
	II	44	19	1	0
	III	28	8	0	0
	IV	40	17	6	4
Small	I	17	7	4	1
	II	18	5	3	1

Nasal spraying proved an effective treatment for the established carriers; and, whereas fifty-one out of 233 'uncontrolled' patients became significant carriers after admission, only one patient (aged 82) out of forty-four became a carrier during the period of nasal disinfection. Nevertheless, only one patient out of sixty-five became a nasal carrier during the periods of skin disinfection. Apart from the carriage of *Staph. aureus*, three nasal carriers of *Pr. mirabilis* were seen (one on admission). 'Soframycin' nebulizers were used unsuccessfully by two of these—despite *in vitro* sensitivity of the causative strains. A nasal carrier of *Ps. pyocyanea* was also encountered after admission.

Ward staff

Serial examinations were made of forty-nine members of the staff; twenty-one were persistent nasal carriers. Four became carriers during the study, and two others became transiently super-infected. No other member of the staff carried the prevalent ward strains of *Staph. aureus*. One of the medical staff suffered a severe attack of sycosis barbae (with his own non-prevalent strain of *Staph. aureus*), and a nurse developed subacute paronychia from which *Pr. mirabilis* was isolated (identical with a ward strain prevalent at the time).

The incidence of hand contamination by *Staph. aureus* and Gram-negative bacilli was very high. Nearly 60 % of random hand impressions yielded pathogens during 'uncontrolled' stages of the work. Hand washing was, however, performed with reasonable regularity. The high incidence seemed to reflect rather the heavily vitiated environment in which fresh contamination was readily acquired. During the stages when skin disinfection was performed, very few examinations of hands gave positive results.

Prevalent pathogens

Over the whole 2-year period, three strains of *Staph. aureus* predominated, each of phage group III. One was confined to the male ward, and had the lytic pattern 47/53/75. It was resistant to sulphonamides, penicillin and tetracyclines, but was sensitive to streptomycin, chloramphenicol, erythromycin, methicillin, fusidic acid, neomycin and the related antibiotic, framycetin. One of the two other strains—both endemic in the female ward—was type 7/47/54/75/81 with the same antibiogram as the previous strain except that it was sensitive to penicillin. The third strain was lysed only by group III phages at 1000 times the routine test dilution, and was resistant to sulphonamides, penicillin, streptomycin, tetracyclines and erythromycin. Both penicillin-resistant strains produced yellow pigment (Willis & Turner, 1962); the sensitive strain was cream-coloured. It was interesting to find that the prevalent strain in the male ward became re-established after an apparent absence of 4 months during the period of skin disinfection. One possible source was the skin of a patient who was readmitted after several months with lesions still infected by this particular strain.

Each of the main wards had a predominant strain of *Ps. pyocyanea*. In the female ward representative strains from lesions and the environment were, with two exceptions, of type 'J', and were resistant to sulphonamides, penicillin, tetracyclines, and erythromycin, but only relatively resistant to chloramphenicol

and streptomycin. In the male ward, type 'M' predominated, and this was resistant to all of the above agents. All strains isolated in this study were sensitive to polymyxin and colistin ('Colomycin').

The differentiation of strains of *Pr. mirabilis* by the technique of Dienes (1946), together with the results of antibiotic sensitivity tests, indicated that several different strains were involved in each of the main wards.

DISCUSSION

The lesions in most dermatological conditions provide a very sensitive indicator of bacterial contamination in the environment. The ease with which infection develops is probably shared only by the comparable lesions of burns (Lowbury, 1960). Thus, 109 of the 233 patients to whom anti-bacterial measures were not applied developed infected lesions after admission to hospital. Primary cross-infection accounted for 53 % of the cases, and super-infection for a further 34 %. The remaining fifteen cases formed a small but significant group in whom autogenous infection occurred.

Duguid & Wallace (1948), and later workers, have shown the facility which which nasal carriers of *Staph. aureus* contaminate themselves and their surroundings. Amongst patients with skin diseases who are nasal carriers, autogenous infection is probably facilitated in hospital, where the patients lie for relatively long periods in self-contaminated bedding. Access of environmental bacteria to the skin is further assisted both by the considerable periods of complete undressing (before and during ward rounds, and during the twice-daily dressing rounds), and by the wearing of scanty night-attire at all other times.

Nasal disinfection was shown by Gould & Cruickshank (1957) to be effective in preventing staphylococcal skin lesions amongst nasal carriers outside hospital. Stratford, Rubbo, Christie & Dixson (1960) recommended the use of a framycetin nasal spray for the treatment and prevention of nasal carriage in hospital, and the present work confirms the value of this procedure. Its routine use in dermatological practice is justified by a reduction in the incidence of autogenous infections. Nevertheless, nasal disinfection had no effect upon the vicious circle of environmental contamination and cross-infection encountered in this study. The subsidiary part which nasal carriage was shown to play in hospital-acquired infection is in agreement with the findings of Henderson & Williams (1963). They showed in a study of 100 surgical patients that nasal carriers had an incidence of wound sepsis similar to that of non-carriers. These investigators considerably modified their interpretation of earlier work (Williams *et al.* 1962) which had seemed to indicate that nasal carriage—present on admission or acquired in hospital—was an important precursor of wound infection.

The success of skin disinfection in breaking the formidable vicious circle of infection focuses attention upon the central role of infected lesions in the dispersal of pathogenic bacteria. The importance of individual 'dispersers' has recently been shown in three branches of hospital practice. Eichenwald, Kotsevalov & Fasso (1960) detected, in a maternity nursery, 'cloud babies' who contributed

large numbers of *Staph. aureus* to their environment when suffering from adenovirus infections of the respiratory tract. Hare & Cooke (1961), and Cooke & Buck (1963) studied small numbers of patients with infected dermatological lesions, and showed that these were responsible for high levels of contamination in their immediate surroundings. Thomas & Griffiths (1961) also obtained their highest air counts of *Staph. aureus* in two small wards devoted to skin diseases. Noble (1962), however, detected in surgical wards, over a 4-year period, only eight dispersers amongst 3675 admissions. The present work provides abundant evidence of the presence of prolific dispersers in dermatological wards, notably with the demonstration of 'broadcasts' of *Staph. aureus* in the bathrooms and wards, together with the high counts of pathogens found in bedding, settled dust, and other situations—especially in the communal baths.

It was shown that these baths, which were used in succession by most of the patients each morning, could be adequately cleansed and disinfected by the method of Boycott (1956). The conditions of the test were particularly exacting in view of the thick greasy applications commonly required in dermatological practice. Because of these dressings, too, the alternative possibility of using showers was not practicable. Another suggestion for countering contamination of baths is to add 1 oz. of 10% hexachlorophene solution (in spirit) to the actual bath water before use (Ayliffe, Alder & Gillespie, 1959). This method was not tried, but, under the difficult conditions described, the similar procedure of adding cetrimide to the water proved valueless (and perhaps even acted selectively for the persistence of *Ps. pyocyanea* and other Gram-negative bacilli).

Despite satisfactory bath hygiene it was disappointing to see no obvious reduction in the incidence of cross-infection (especially with the Gram-negative organisms); evidently numerous additional routes were involved. Thus, surprising reservoirs of Gram-negative bacilli were found in ward dust, indicating frequent, fresh contamination—presumably from the skin lesions of patients. Apart from the heavily contaminated air, further hazards to the patients were provided by the attendants' hands, which gave alarming counts of pathogens—often shortly after washing. Nasal carriage by members of the staff did not, however, seem to be of any importance in the spread of infection. Indeed, it was interesting to find that the staff, in contrast to patients, rarely became carriers of the prevalent pathogens. This finding may be connected with the relatively short duration of exposure to the ward environment.

Recently, convincing evidence has been advanced to suggest that two vehicles are chiefly concerned in dust-borne infection. Rubbo, Pressley, Stratford & Dixson (1960) favour 'fibre nuclei'—fragments of cellulose fibres about 15 μ in diameter. Davies & Noble (1962), working in similar environments, believe that epidermal scales of equivalent size are more important. In the present work cellulose certainly constituted the majority of fibres found in infected dust samples. However, a large part of the dust was composed of keratin material and was often seen to consist of microscopic epidermal fragments. Undoubtedly, both vehicles are involved in a dermatology department, but evidence is at present accumulating to show the importance of epidermal debris in the transmission of infection. Direct measure-

ments in individual skin conditions (to be reported) show that subclinically infected patients, notably those with psoriasis, are particularly dangerous sources of such contaminated debris.

The systematic use of antibiotic sprays on lesions effectively solved the problem of the disperser. The low incidence of nasal carriage observed after skin disinfection might simply have been due to the marked reduction in environmental contamination, but a direct effect on the anterior nares by aerial dissemination—such as Elek & Fleming (1960) produced by spraying methicillin in a ward—cannot be ruled out.

Despite the justification for their use, the potential dangers of such sprays must be considered. In this study neither selection of resistant organisms nor sensitization phenomena in patients were encountered. In view of a recent report from another part of Scotland (Robertson, 1963) a particular watch was kept for signs of the emergence of neomycin resistance in *Staph. aureus*. The use of a mixture of unrelated bactericidal antibiotics in this work undoubtedly militated against the development of neomycin resistance in the prevalent group III strains. The particular antibiotics chosen are—with the exception of colistin—unsuitable for systemic use, and can therefore be reserved for topical application. Colistin is, however, sometimes administered parenterally in the form of the methane sulphonate. Unfortunately the colistin–polymyxin group of antibiotics is the only one presently available which can deal adequately with the ubiquitous *Ps. pyocyanea*. In an attempt to avoid the use of topical antibiotics entirely, tests are now being carried out upon an interesting new agent—'polynoxylin' (Annotation, 1963)—in the form of an aerosol.

During the relevant stages of the present work, all lesions were sprayed. This was because of the high and rising incidence of infection present at the outset. It is not, however, suggested that prophylactic anti-bacterial agents should be used routinely in dermatological conditions; but the early recognition and elimination of clinical or subclinical infection in skin lesions is essential if gross environmental contamination is to be prevented. It is hoped that current work will indicate those patients who constitute the greatest potential hazard in hospital, and who therefore require special attention.

SUMMARY

Over a 2-year period a dermatology department was shown to be a valid model for the long-term study of hospital cross-infection. In the absence of specific anti-bacterial measures, consistently high levels of *Staph. aureus* and Gram-negative bacilli were found throughout the environment. 'Broadcasts' of *Staph. aureus* were particularly evident in the bathrooms, and notable sites of heavy, cumulative contamination were the communal baths, bedding, settled dust, and the hands of the staff.

Cross-infection of lesions occurred in ninety-four of the 233 'uncontrolled' patients—15 cases being due to Gram-negative bacilli. Autogenous infection occurred in another fifteen cases. Nasal carriage of *Staph. aureus* developed in fifty-one patients after admission.

In one of the two main wards nasal disinfection (with 'Soframycin' nebulizers) was used for 4 months, and was effective in the treatment and prevention of nasal carriage; but it produced no change in the level of environmental contamination or in the incidence of cross-infection—even though efficient cleansing of the baths was also instituted. In contrast, during two separate periods of the work disinfection of skin lesions (with 'Rikospray Antibiotic') markedly reduced the counts of pathogens in the environment, and virtually abolished both cross-infection and the development of nasal carriage.

I am grateful to Prof. G. H. Percival and Dr G. A. G. Peterkin for allowing free access to their wards at all times. Dr J. C. Gould kindly carried out phage typing of strains of *Ps. pyocyanea*.

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Elimination of *Eperythrozoon coccoides* infection from mouse colonies

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During the past 15 years the importance of *Eperythrozoon coccoides* Schilling, 1928, in certain infections of mice has been amply demonstrated. Infection with *E. coccoides* does not itself produce overt signs of ill-health, but it causes splenic hyperplasia and alters the responses of mice to other agents, sometimes with fatal results. Experimentally *E. coccoides* enhances the pathogenicity of mouse hepatitis virus so that fatal hepatitis ensues (Niven, Gledhill, Dick & Andrewes, 1952; Gledhill & Dick, 1955). *E. coccoides* also increases the pathogenicity of lymphocytic choriomeningitis virus inoculated peripherally, converting a mild immunizing infection into a fatal disease (Seamer, Gledhill, Barlow & Hotchin, 1961). Similarly, the parasite can increase the susceptibility of mice to the lethal action of Gram-negative bacteria and their endotoxins; during the phase when eperythrozoa are abundant in the blood, mice are about 100 times as susceptible to bacterial endotoxin as normal mice (Gledhill & Niven, 1957). Infection with *E. coccoides* increases the lactose dehydrogenase activity of the blood plasma in a way similar to the Riley agent (Arison, Cassaro & Shouk, 1963). The infection has also been shown to increase the phagocytic activity of mice, as judged by the uptake of intravenously inoculated carbon particles (D. L. J. Bilbey, A. W. Gledhill & J. S. F. Niven, unpublished data).

Since *E. coccoides* can readily be transmitted from infected mice by inoculation of blood, or blood-containing tissues, this potentially serious source of error should be eliminated from mice used for experimental purposes. Like other eperythrozoa, *E. coccoides* is believed to be transmitted under natural conditions by biting insects and particularly by lice (Eliot, 1936). In infected breeding colonies, experience has shown that the majority of mice become infected within the first months of life, and after a week or so during which parasitaemia is readily detected, infection may persist for 6 months or longer. The purpose of this communication is to show that *E. coccoides* infection can be eliminated from a mouse colony by regular insecticidal treatments designed to reduce infestation with the mouse louse, *Polyplax serrata*, to a very low level, if not to eradicate it completely.

MATERIALS AND METHODS

Mice. The breeding colony of Parkes (P-strain) mice has been maintained as a closed colony at the National Institute for Medical Research, Hampstead and Mill Hill, since 1922 and is known to have been infected with *E. coccoides* for more than 10 years. Lice were occasionally observed on P-strain mice before the work

to be described. In testing for the presence of *E. coccoides* in P-strain mice, mice of the TO-strain were used. This strain has been bred as a closed colony at the Institute since 1953 and was found not to be infected with *E. coccoides*.

Insecticidal treatment

A powder containing pyrethrum and piperonyl butoxide ('Pybuthrin', Cooper, McDougall and Robertson, Ltd.) was blown into each of the mouse boxes containing P-strain mice twice weekly for 6 weeks and thereafter once every two weeks.

Estimation of the proportion of mice infected with Eperythrozoon coccoides

Infection with *E. coccoides* is readily detected by examining blood smears taken daily for some 10 days after splenectomy of individual mice. However, in order to facilitate the examination of a representative proportion of the colony, an indirect method based on the enhancing effect of *E. coccoides* upon mouse hepatitis virus was used, the enhancement being recognized by fatal hepatitis or by degrees of liver damage greater than in normal mice inoculated with virus alone. Ten groups of ten P-strain mice aged 21 days were taken at random from the colony. (At this age it had been our general experience that about 25 % of mice are infected, whereas at 10–12 weeks of age 50–100 % are infected.) The mice were killed and the spleens from each group were removed and emulsified in 10 ml. of chilled serum saline broth (10 % horse serum, 45 % beef infusion broth, 45 % normal saline). After light centrifugation, 0·2 ml. inocula of the supernatants were injected intraperitoneally into groups of five TO-strain mice aged 18–21 days. On the following day the mice received an inoculation of about 100 ID50 mouse hepatitis virus (MHV 1); a control group of mice which received virus only was included in each experiment. All mice that died were autopsied and 6 days after the virus inoculation the surviving mice and the controls were killed and autopsied. Macroscopic liver lesions were scored according to a scheme described by Gledhill (1961) as follows:

Appearance of liver	Score
No obvious focal lesions	0
A few focal lesions	1
Many focal lesions	2
Coalescence of focal lesions to give generalized liver abnormality	3
Liver bright yellow with haemorrhagic areas	4
Mouse dead with liver as in last group	5

Enhancement of MHV 1 as evidenced by severe macroscopic liver damage (score 3 or greater) in most mice of a group was taken to indicate the presence of *E. coccoides* in at least one of the P-strain mice that provided the inoculum of spleen suspension; and in the absence of such damage it was considered that none of the P-strain mice was infected with *E. coccoides*. With these assumptions it was possible to give an estimate of the proportion of mice infected with *E. coccoides* among the 100 examined. This procedure was carried out when treatment began and after 6, 15 and 30 weeks.

RESULTS

The results of the first test, made when treatment began, are shown in the upper half of Table 1. On the basis that inoculation of MHV 1 into normal mice caused liver lesions that scored two or less, it is apparent that the pathogenicity of the virus was enhanced in nine groups of mice, and only one group was considered to be free of infection with *E. coccoides*. Calculations based on the binomial distribution show that an infection rate of 21 % among the 100 P mice examined would

Table 1. *The incidence of* Eperythrozoon coccoides *infection in weanling P-mice* (*a*) *before and* (*b*) *after* 6 *weeks of insecticidial treatment*

Groups of five weanling mice	Liver score at autopsy 1	2	3	4	5	Total	*E. coccoides* infected
			(*a*) Before treatment				
1	1	1	1	0	0	3	0
2	5	4	3	3	0	15	+
3	4	4	4	3	2	17	+
4	4	3	3	3	0	13	+
5	4	4	4	3	3	18	+
6	5	5	5	5	3	23	+
7	5	4	4	4	0	17	+
8	5	5	3	3	1	17	+
9	4	4	4	3	0	15	+
10	4	4	4	4	0	16	+
			(*b*) After 6 weeks treatment				
1	5	5	4	4	0	18	+
2	1	1	0	0	0	2	0
3	5	5	4	4	3	21	+
4	2	1	0	0	0	3	0
5	2	2	1	0	0	5	0
6	2	2	1	0	0	5	0
7	1	0	0	0	0	1	0
8	5	4	4	4	2	19	+
9	2	0	0	0	0	2	0
10	2	1	0	0	0	3	0

(1) Each group of five weanling TO mice inoculated i.p. with 0·2 ml. 10 % spleen suspension from ten weanling P-mice and 1 day later 0·2 ml. i.p. liver suspension containing about 100 ID 50 MHV 1.

(2) Mice which died autopsied and scored for hepatitis. Survivors at 6 days after virus inoculation killed, autopsied and likewise scored.

(3) The system of scoring liver damage is stated under methods.

be most likely to give this result and would in fact give it in 39 % of tests. If, however, the actual infection rate was less than 7 % or greater than 50 % the result obtained would be expected in less than 1 test in 100. These inferences accord also with the observation that twenty-two spleens from the original 100 P mice were considered to be enlarged, while the remainder were considered to be of normal size or small.

The second test was carried out at the end of the 6-week period of bi-weekly treatment, and the results appear in the lower half of the table. A clear distinction was again apparent between three groups in which the pathogenicity of MHV 1 was enhanced and seven groups in which it was normal. Similar calculations show that an infection rate of 3·5 % would be most likely to give the result obtained, and would in fact give it in 27 % of tests. If the actual infection rate was less than 0·5 % or greater than 11 % this result would be expected in less than 1 in 100 tests.

The third and fourth tests were carried out 9 and 24 weeks after the second test, during which time the treatment was applied once fortnightly. In both these tests the pathogenicity of MHV 1 was within normal limits in all groups, and it was inferred that none of the donors were infected with *E. coccoides*.

The results suggested that treatment against lice had prevented the spread of *E. coccoides* to mice born after the treatment was started. In order to obtain further evidence that infection with *E. coccoides* was no longer present in the colony, twenty-one female P-strain mice aged about 12 weeks were selected at random after treatment had been given for 1 year. These mice were splenectomized, and blood smears were taken from each on the 3rd, 6th, 7th, 8th, 9th, 10th, 13th and 15th days after operation. No eperythrozoa were seen in the 168 blood films examined.

DISCUSSION

We assume from these results that after 6 weeks of intensive and 9 weeks of fortnightly insecticidal treatment, the spread of *E. coccoides* to mice born within the infected colony had been stopped. No doubt some older mice were still infected with eperythrozoa at this time, for Derrick, Pope, Chong, Carley & Lee (1954) found that infection persisted for 4 months in some mice, and we have experimental evidence to support this finding. However, although some mice are retained in the colony for breeding purposes for as long as 9 months, there appeared to be no spread from the long-term carriers, for the splenectomies performed 1 year after treatment began failed to reveal infection with *E. coccoides* in mice of an age group in which the incidence would previously have been 50–100 %.

We have evidence that large doses of *E. coccoides* will infect a proportion of mice when given *per os* (J. Seamer, unpublished observations), and transmission by this method might possibly become significant in colonies in which deaths were frequent and cadavers often eaten, but this was not the case in the P colony. As to the ectoparasites concerned, it is known that *E. coccoides* can be transmitted by lice (Eliot, 1936; G. W. A. Dick, personal communication), and lice had been observed on the P mice. Fleas were not observed, but in any event the insecticide should have been as effective against fleas as against lice. Mites which have been observed on the mice were unlikely to be responsible for the maintenance of infection since the insecticide is not effective against them. Although *E. coccoides* seems to have been eliminated from our colony, we do not assume that lice have been entirely eliminated; it is possible that reduction of the louse infestation below a critical level would effectively curtail the transmission of the eperythrozoa.

The use of MHV 1 to indicate the presence of *E. coccoides* enabled the proportion

of infected mice at the various stages of treatment to be estimated more quickly than by the direct method of splenectomy and examination of blood smears. However, certain murine leukaemia agents also enhance the pathogenicity of MHV 1 (Gledhill, 1961) and inferences based upon results obtained with the virus require at some point to be confirmed by observations of blood smears.

The necessity of providing mice free of *E. coccoides* for experimentation has been emphasized and, on the basis of our experience, it would not appear difficult to provide such mice. Measures to control lice by insecticidal treatment should be carried out as a routine in all conventional colonies. Suspected infection with *E. coccoides* in a colony should be proved by the daily examination of blood smears for 10 days from a dozen or so splenectomized mice. If infection is demonstrated the test should be repeated after about 6 months with another group of mice, to assess the effect of prophylactic measures. For these tests mice about 3 or 4 months old are most suitable since an infection rate representative of the colony will not have developed in very young mice and will be more difficult to demonstrate with increasing age. In this connexion the fact should be stressed that *E. coccoides* is readily transmissible to intact mice and that parasitaemia is easily demonstrable; indeed, we have utilized this characteristic of *E. coccoides* as the basis of much experimental work over many years (Niven *et al.* 1952; Seamer, 1959).

SUMMARY

1. Long-established infection of a mouse colony with *Eperthyrozoon coccoides* was eliminated by regular insecticidal treatments designed to reduce infestation with lice and fleas.

2. Undesirable consequences of *E. coccoides* infection upon mice used as experimental animals are noted and an easy routine for exclusion of this parasite from mouse colonies is suggested.

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The incidence of infection with cytomegalovirus in a normal population

A serological study in Greater London

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Disease caused by cytomegaloviruses occurs in its most severe and characteristic form in the neonatal period. The majority of these cases acquire their infections *in utero* and the clinical features, which are present at birth or appear shortly afterwards, include jaundice with hepatosplenomegaly, thrombocytopenic purpura, erythroblastic anaemia, pneumonitis and often evidence of neural damage which may be associated with microcephaly and cerebral calcification. This condition was previously believed to be almost always fatal, but it is now known that recovery does occur although it may be followed by residual abnormalities such as mental deficiency, microcephaly, hydrocephalus, epilepsy, cerebral palsies and optic atrophy (Birdsong, Smith, Mitchell & Corey, 1956; McElfresh & Arey, 1957; Weller & Hanshaw, 1962; Medearis, 1964). When older infants are infected the clinical pattern is ill-defined and usually takes the form of an unresolving interstitial pneumonia or sometimes intractable gastro-intestinal symptoms, often with evidence of hepatic or renal dysfunction (Smith & Vellios, 1950; Wyatt, Sexton, Lee & Pinkerton, 1950; Medearis, 1957). These post-natal infections tend to be superimposed on underlying serious debilitating diseases. The true incidence of the neonatal and post-natal diseases is uncertain, since many of the fatal cases and of those that recover are undiagnosed. Fatal cases, however, have accounted for 1–2% of unselected paediatric autopsies in Boston and St Louis in the United States, as well as in Finland and Germany (Farber & Wolbach, 1932; McCordock & Smith, 1934; Wyatt *et al.* 1950; Ahvenainen, 1952; Seifert & Oehme, 1957). In other parts of the United States and also in Great Britain they seem to be very much less common (Potter, 1957; Symmers, 1960; Crome & France, 1959).

After 4 years of age the disease is very rare. Most cases, in children and adults, have been recognized unexpectedly at post-mortem or in biopsy specimens taken for other purposes (Wong & Warner, 1962). It occurs mostly as a complication of underlying chronic debilitating conditions such as leukaemia and lymphoma which depress the normal defence mechanisms of the body, especially when steroids and cytotoxic drugs have been used in treatment.

Although clinical disease is infrequent it has been known for some time that a more common but inapparent or symptomless form of infection occurs in early childhood. This is revealed by the presence of typical cytomegalic cells in the

salivary glands and occasionally also in the kidneys. Such cells are an incidental finding at post-mortems carried out on children who have died from other causes, and are rarely seen under 2 months or after 2 years of age. They have been described in 10–12% of unselected paediatric autopsies in Germany and the United States (Löwenstein, 1907; Seifert & Oehme, 1957; Farber & Wolbach, 1932; McCordock & Smith, 1934) and in as many as 18 and 32% of autopsies in South America and Indonesia respectively (Potenza, 1954; Prawirohardjo, 1938). In Great Britain the incidence has been 5% or less (Baar, 1955; McDonald, personal communication). Serological studies, which became possible after the isolation of cytomegaloviruses in tissue culture (Rowe *et al.* 1956; Smith, 1956; Weller, Macauley, Craig & Wirth, 1957), have confirmed that infection as opposed to disease is common in children. They have also shown infection to be common in adults, the incidence of antibodies increasing progressively with age to as high as 81% after 35 years of age in the Washington area (Rowe *et al.* 1956).

The present paper is a serological study of infection in the London area, using the complement fixation method.

METHODS

Virus strains. These were 'Kerr', 'Davis' (Weller *et al.* 1957) and 'Ad 169' (Rowe *et al.* 1956) isolated in the United States, and 'Aravi', '138', 'Rawles' and 'Sh' isolated in this country (Stern, Lambert & Shakespeare, 1963). They were grown in human embryonic fibroblast tissue cultures. In the early part of the investigation these cultures were prepared as primary outgrowths in plasma clot; later semi-continuous diploid cell lines were used (Hayflick & Moorhead, 1961). The plasma clot cultures were incubated in roller tubes at 37° C. and both the growth and maintenance media were Earle's saline containing 0·5% lactalbumin hydrolysate, 10% inactivated horse serum, 0·01% soy bean trypsin inhibitor and 200 units per ml. each of penicillin and streptomycin. The pH was adjusted with 5% sodium bicarbonate to about 7·2. The diploid cultures were grown in stationary tubes in Eagle's medium with 10% newborn calf serum and antibiotics and bicarbonate as above, and then maintained on medium 199 with 2% newborn calf serum. The viruses were passaged by scraping infected cells off the glass into the medium, grinding the cell suspension for 15–20 sec. in a Ten Broek grinder and inoculating 0·1 ml. of ground suspension into fresh tissue culture tubes. Tissue culture-adapted strains, when kept in continuous passage without intervening storage, caused complete destruction of the cell sheet in 5–10 days. Less well-adapted strains required as long as 21–28 days or more.

Complement-fixation tests. Antigens were prepared by inoculating tubes containing fully grown sheets of fibroblasts with 0·1 ml. of ground infected cell suspension. The maintenance medium was changed after 2 days and subsequently usually at 3-day intervals. When the sheets showed almost complete cytopathic effects the cells were scraped into the medium. Pooled cell suspensions were centrifuged at 1500 r.p.m. for 10 min. and the deposited cells resuspended in veronal-buffered saline to one-fifth of the original volume. The cells were then disrupted by thorough grinding in chilled glass grinders or by exposure for 5 sec.

to ultrasonic vibrations. The treated suspension constituted the antigen and was kept frozen at −70° C. in 1 ml. amounts in neutral glass ampoules. Just before use the antigen was heated at 56° C. for half an hour. Uninoculated tubes from the same batch of tissue culture were treated in the same way for use as controls. Each batch of antigen was examined in a chess-board titration against a standard positive pooled human serum. The antigen was used in the C.F. test at a dilution containing 2 units per 0·1 ml. Most preparations contained 2 units at a dilution of 1 in 2 or 1 in 4 and were free of anticomplementary activity at these dilutions. They maintained their potency at −70° C. for at least 1 month. Latterly, the infected tissue culture cells were resuspended in veronal-buffered saline containing 25 % sorbitol before disruption. Such preparations retained unaltered C.F. activity for longer periods (Medearis, personal communication).

In the C.F. test all sera were inactivated at 56° C. for half an hour. Tests were carried out in Wasserman tubes, using 2 units of complement and overnight fixation at 4° C. Sera were first examined at a dilution of 1 in 8, and if positive were titrated. A serum was regarded as positive when it showed in the test at least 75 % fixation. Any specimen showing less than this reaction was considered doubtful and was retested at 1 in 4 dilution. Only if it then gave 75–100 % fixation was it regarded as positive.

RESULTS

Blood specimens were obtained from individuals of various ages as shown in Table 1. The children under 10 years old were in-patients mostly with minor illnesses at three children's general hospitals; blood was taken on admission or shortly afterwards. The 10- to 15-year-olds included seventy-five children from the same hospitals and from an orthopaedic hospital, and 182 healthy children from two mixed day schools. The older age groups comprised healthy persons who had blood taken for tests needed for travel. Socio-economic status was not investigated. The table illustrates the incidence of C.F. antibodies in the different age groups, using the 'Kerr' strain of virus as antigen.

Table 1. *Incidence of cytomegalovirus complement-fixing antibodies*

Age groups (years)	No. positive / No. tested	% positive
0–½	3/9	33
½– 5	4/93	4
5–10	15/97	15
10–15	54/257	21
15–25	47/130	36
25–35	62/114	54
35–75	46/85	54

The positive results obtained in infants under 6 months old are probably due to maternal antibody since, although the numbers are small, the incidence is closely similar to that of the adult child-bearing group. During the pre-school period, 6 months to 5 years, infection is apparently infrequent with only 4 % of children possessing antibodies. After 5 years antibodies become increasingly prevalent with age to reach a maximum incidence of 54 % by 25–35 years of age. This level is then

maintained in the older age groups. No significant sex differences were noted. Table 2 shows the antibody titres of the positive sera and the geometric mean titres for each group.

The majority of the subjects in the above study were obtained at random from the general population, and included two groups of 10- to 15-year-old children attending two separate day schools. These were later compared with boys of the same age from a boarding school in which the opportunities for cross-infection are presumably greater (the latter were not included in the totals described in Table 1). Table 3 shows the strikingly higher incidence of antibodies in the boarding school population.

Table 2. *Distribution of titres of sera possessing complement-fixing antibodies*

Age group (years)	No. tested	No. of sera having indicated titre						Geometric mean titre
		8	16	32	64	128	256+	
½– 5	4	—	—	2	—	—	2	90
5–10	12	2	—	—	3	—	7	97
10–15	53	2	1	24	6	18	2	56
15–25	47	8	—	21	9	4	5	40
25–35	62	12	—	44	—	6	—	28
35–75	46	9	—	18	6	3	10	46

Table 3. *Incidence of complement-fixing antibodies in three school populations*

School	No. positive / No. tested	% positive
Day 1	35/121	29
Day 2	15/83	18
Boarding	40/50	80

Specificity of the complement-fixation test

Cross-C.F. tests were carried out using seven strains of cytomegalovirus. The three American strains, 'Kerr', 'Davis' and 'Ad 169', have been shown to represent three distinct serotypes on the basis of the neutralization test (Weller, Hanshaw & Scott, 1960). The London strains were 'Aravi' isolated from a 6-month-old child with a malignant giant haemangioma, 'Sh' isolated from an apparently healthy 7-day-old baby who was found to have an enlarged liver and spleen and a reduced platelet count, and '138' and 'Rawles' isolated respectively from a fatal case of neonatal cytomegalic inclusion disease and from a 4-year-old boy with symptomless hepatomegaly and abnormal liver function tests (Stern *et al.* 1963; Stern & Tucker, unpublished). Since it has not yet proved possible to prepare animal antisera against these strains of virus they were tested against human sera from cases of current or recent infection. These were available from the three infants from whom strains 'Aravi', 'Sh' and 'Rawles' were isolated. Sera were also obtained from their mothers, who were found at the time not to be excreting virus, and from a 4- and a 2-year-old sibling of the 'Aravi' and 'Sh' cases respectively. The former was excreting virus without symptoms. Virus was also isolated

from the other child, who was found to have grossly abnormal liver function tests but was clinically well. An additional serum tested was from a woman (Ogg.) who had given birth to a fatal, histologically confirmed case of neonatal disease 1 year previously. The C.F. antibody titres of these various sera as determined with antigens prepared from each of the seven virus strains are shown in Table 4. Each serum cross-reacted with all seven viruses, and no evidence was obtained of significant antigenic differences among these strains.

Table 4. *Complement-fixation tests with sera from recent infections and seven strains of cytomegalovirus*

	Antigen						
Serum	Kerr	Ad 169	Davis	Aravi	138	Sh	Rawles
Aravi	128*	128	64	128	32	64	256
mother	64	64	64	128	64	64	256
sibling	64	64	64	64	64	64	128
Sh	32	32	16	8	8	16	32
mother	64	64	64	16	16	64	128
Rawles	32	128	32	16	16	32	64
mother	128	64	32	16	8	8	64
sibling	32	32	32	8	8	16	32
Ogg.	64	64	64	64	64	64	128

* Serum titre.

A number of positive and negative sera from the normal population study were re-examined, at 1 in 8 dilution, in a single C.F. test against the seven strains of viruses. These comprised twenty-five negative sera from each of the 5–10, 10–15 and 25–35-year-old age groups, and 15 positive sera each from the 5–10 and 25–35-year groups. Previously 100 negative sera and 40 positive sera from 5–15-year-old children in an institution (Stern & Elek, unpublished) had been examined simultaneously with strains 'Kerr', 'Ad 169' and 'Davis'. With every serum in these tests identical positive or negative results were obtained with all the virus strains used.

These various findings would appear to indicate that the seven strains of cytomegalovirus, isolated from the widely separated areas of London and the United States, have common group-specific C.F. antigens. Although many more strains require to be examined before it can be concluded that all cytomegaloviruses possess a common group antigen, the use of a single strain in an epidemiological survey seems to be justified.

Serum reactions with control antigen prepared from uninoculated human embryonic fibroblasts have been observed only three times in well over 1500 serum tests. One serum was from a 2-year-old mentally retarded child from a serological study of cytomegalovirus infection in relation to mental deficiency (Stern & Elek, unpublished). The other two sera were both from women who had recently given birth to infants with neonatal hepatitis. Cytomegaloviruses were not isolated from the urine or throats of the infants and a specific diagnosis could not be made in either case.

DISCUSSION

In many virus infections the C.F. antibody response is transient, while neutralizing antibodies persist for many years. However, in other infections such as those caused by the herpes viruses C.F. antibodies persist for life, and can therefore be used for an epidemiological survey of past infection. This also applies to the closely related cytomegaloviruses (Rowe *et al.* 1956).

Infection with cytomegaloviruses, as opposed to clinical disease, is obviously widespread in London. The incidence, based on our serological findings, appears to be low in early childhood since only 4% of pre-school children have demonstrable antibodies. This agrees with the infrequent finding of cytomegalic cells in the salivary glands of unselected paediatric autopsies in this country (Baar, 1955; McDonald, personal communication). The incidence of infection mounts steadily through the school-age period and adult life to reach its maximum of 54% by 25–35 years. Young adults are, therefore, apparently exposed as much to infection as school-children. It is probable that children are the main source of infection since it has been shown that they may excrete virus in their mouths, as well as in the urine, for prolonged periods (Rowe, Hartley, Cramblett & Mastrota, 1958). There is little information concerning the excretion of virus in infected adults, although it has been demonstrated that women who have given birth to babies with cytomegalic inclusion disease may continue to excrete virus in their saliva for many months without symptoms (Medearis, 1964). However, the importance of adult carriers in the spread of infection is as yet unknown. The fact that the incidence of C.F. antibodies does not increase after the age of 35 suggests that older individuals are less exposed to infection, perhaps because there is less intimate contact with small children.

The persistence of C.F. antibodies in the population suggests, by analogy with herpes simplex, that after recovery from the primary infection the virus is not eliminated from the body but persists in a latent form. Activation of such latent infection occurring as a complication of diseases which depress the immunity mechanisms of the body has been invoked to explain the rare disseminated disease in adults (Nelson & Wyatt, 1959); although it is also possible that this follows exogenous infection and that dissemination results from the failure of immunity. At present there is no evidence on whether intermittent reactivation with excretion of virus occurs in healthy individuals, as it does in herpes simplex.

The low rate of infection among school-children indicates that the virus does not spread easily and that close and prolonged contact may be necessary for cross-infection. Thus, among 83 children attending a day school only 18% had cytomegalovirus antibodies as compared with a 45% incidence of herpes antibodies. The increased rate of infection with closer prolonged contact is shown by the 80% incidence of cytomegalovirus antibodies in the boarding school, and high infection rates have also been demonstrated among institutional children and family contacts (Hanshaw & Simon, 1962; Weller & Hanshaw, 1962; Stern *et al.* 1963). Cytomegalovirus is more labile in the extracellular environment than the herpes virus and this probably accounts for the low degree of contagion.

Our findings at all ages differ considerably from those of Rowe and his colleagues

(1956) in Washington, who found C.F. antibodies in 30% of 5-year-olds and 81% of adults over 35 years. It seemed possible that our lower figures were due to the use of a heterotypic strain of virus whereas Rowe *et al.* employed a strain (Ad 169) isolated in the Washington area. However, the re-examination of sera with antigens prepared from various viruses isolated in the United States, and locally in London, shows that this is not the explanation. Our finding of common group-specific C.F. antigens is in agreement with that of Medearis (1964), who also showed that although the early C.F. antibody response in neonatal disease may tend to be strain specific it later becomes group specific. The more likely explanation of the differences in antibody prevalence between London and Washington is to be found in the socio-economic status of the two populations tested. Differences in antibody incidence have previously been demonstrated in small children of various countries, ranging from 15% in Virginia to 85% in Egypt (Rowe, 1960). In the same way, cytomegalic cells have been found in the salivary glands of 10–12% of unselected paediatric autopsies in Europe and North America (Farber & Wolbach, 1932; McCordock & Smith, 1934; Seifert & Oehme, 1957) as compared with 18% and 32% in the less highly developed areas of South America and Indonesia (Potenza, 1954; Prawirohardjo, 1938). As with poliovirus infections, improved standards of hygiene reduce the chances of infection in early childhood so that the first striking increase of infection occurs in older children when they come together at school.

In view of the finding that about a third of women become infected and acquire their antibodies during the main child-bearing period (15–35 years of age) it is surprising that neonatal disease is apparently so rare in Britain as compared with other countries. It may in fact be more common than previous reports would indicate. The severe neonatal syndrome is probably often misdiagnosed during life because of unfamiliarity with the disease, and even in fatal cases diagnosis may be possible only by virological methods (Weller & Hanshaw, 1962; Stern *et al.* 1963). In addition, many non-fatal cases undoubtedly occur without the full classical neonatal picture. They may present with only mild hepatosplenomegaly; such cases can be recognized only by means of virus isolation.

After the neonatal period the bulk of infection is apparently subclinical. A proportion of the cases, however, may possibly be associated with minor illness. Women who have produced babies with neonatal disease have sometimes given histories of respiratory illnesses during the course of pregnancy (Weller & Hanshaw, 1962; Medearis, 1964), and the almost invariable presence of lung lesions in both neonatal and adult disease stresses the importance of the respiratory site of entry of the virus. Small children with apparently symptomless excretion of cytomegalovirus in the urine may have hepatomegaly or abnormal liver function tests (Rowe *et al.* 1958; Hanshaw & Simon, 1962). Whether this is always transient or occasionally results in more chronic liver disease is as yet unknown. Disease caused by cytomegaloviruses is clearly more prevalent and has a broader range of symptomatology than previously recognized. A clear picture can only emerge of the lesser forms of illness in children and adults if the disease is kept in mind and virological investigations are used more extensively.

SUMMARY

A serological study of cytomegalovirus infection in London shows it to be prevalent. Under 5 years of age only 4 % of children have antibodies, but this increases to 15 % by 10 years of age and 21 % by 15 years. The maximum incidence of 54 % is reached by 25–35 years, and this is maintained in the older age groups of the population. In two mixed day schools the incidence of infection was significantly lower than in a boarding school, suggesting that close prolonged contact is required for spread of infection.

Different strains of cytomegalovirus possess common group-specific complement-fixing antigens.

It is suggested that clinical disease caused by cytomegaloviruses is more frequent and more varied than previously believed, although diagnosis may be difficult without virological help.

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Toxoplasma skin- and dye-test surveys of severely subnormal patients in Lincolnshire*

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Cerebral damage is one of the four cardinal features of the classical syndrome of congenital toxoplasmosis described by Sabin (1942). Feldman (1958) tabulated the frequency with which the various manifestations were found by Eichenwald in 180 proven cases and showed that psychomotor retardation was present in 45%. Thalhammer (1962) showed that, in addition to these recognizable cases, there was serological evidence that many patients, lacking other features of toxoplasmosis and with otherwise unexplained brain damage, might, in fact, be cases of this disease. He found a 20% excess of positive dye tests in severely subnormal children attending his paediatric clinic in Vienna, as compared with normal controls, and suggested that congenital toxoplasmosis might have caused this proportion of cases. Butler (1959) indicated the need to ascertain serologically the proportion of known defectives whose condition is attributable to toxoplasmosis.

Dye-test surveys of subnormals have been undertaken by Cook & Derrick (1961) in Australia, and by Fleck (1963) in Swansea. These did not suggest that toxoplasmosis was a significant cause of mental deficiency in the populations they studied. Fair (1959), using the skin test in a large series in America, reached the same conclusion. In view of evidence of the prevalence of toxoplasmosis in Lincolnshire (Beattie, 1957; Robertson, 1960, 1962), it was decided to examine all subnormals in hospitals in the county.

METHOD AND MATERIALS

There are four hospitals for the mentally subnormal in Lincolnshire and these were visited in turn. Of 918 patients 917 were subjected to the skin test. At one hospital only those patients who were under the age of 11, or who were positive to the skin test or had epilepsy or eye disease were examined seriologically. At the remaining hospitals the serological testing was extended to include all adult patients in order to establish the comparability of skin and dye tests.

Skin-test antigen was made by Eli Lilly and Co. (U.S.A.) and supplied through their English subsidiary free of charge. The antigen was given by intradermal injection of 0·1 ml. into the left forearm and, initially, mouse spleen control antigen was given in the right forearm. Subsequently, the use of control material was discontinued, as only one patient had shown any reaction to it.

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The tests were read on the third day, the diameter of any area of induration being measured. (Induration exceeding 7 mm. in diameter was taken to indicate a positive result.)

RESULTS

Dye-test results

Of the 717 sera taken from subnormal patients in the four hospitals, thirteen leaked in transit and, of the remainder, forty-nine were from cases selected on account of special clinical features or positive skin test. Table 1 shows the incidence of dye-test positivity in the remaining 655 cases.

In order to assess the significance of these figures it is necessary to compare them with figures for the general population.

Table 1. *Dye-test results in wards where all were tested*

Age groups	−ve	+1/8 −1/32	+1/32 −1/128	+1/128 −1/256	+1/256 −1/512	+1/512 −1/1024	+1/1024 −1/2048	+1/2048 −1/4096	+1/4096 and over	Total	Total +ve	% +ve 1/8
1–9	36	3	1	2	2	2	—	—	1	47	11	23·4
10–19	93	10	12	6	—	1	—	1	—	123	30	24·4
20–29	74	16	24	2	—	—	—	—	—	116	42	36·2
30–39	60	13	17	1	—	—	—	—	—	91	31	34·1
40–49	67	26	21	1	—	—	1	—	—	116	49	42·2
50–59	35	32	31	2	—	—	—	—	—	100	65	65
60+	26	21	12	2	1	—	—	—	—	62	36	58
Total	391	121	118	16	3	3	1	1	1	655	264	40·3

In this and other tables, +1/8, −1/32 signifies positive at 1/8 and negative at 1/32.

Table 2. *Dye-test titres of* 220 *normal children in Lincolnshire*

(Sera tested at Leeds in 1962–63.)

Age groups	−ve	+1/8 −1/32	+1/32 −1/128	+1/128 −1/256	+1/256 −1/1024	+1/1024 −1/2048	+1/2048	Total	Total +ve	% +ve
1–4	171	—	—	1	1	1	1	175	4	2·3
5–9	44	—	—	—	1	—	—	45	1	2·2
Total	215	—	—	1	2	1	1	220	5	2·3

Children

The best estimate available of the incidence in children in the area comes from tests undertaken in the same year on sera I took from children in the north of the county in connexion with another investigation. These sera were tested at the same laboratory, and the incidence of dye-test positivity is shown in Table 2. Only 2·3% of normal children below the age of 10 have positive dye tests, whereas 23·4% of subnormals of the age are positive at a titre of 1/8.

The difference between the percentages positive is 21·1. As the standard error is 3·83, the difference in proportions is $5\frac{1}{2}$ times the standard error and the difference is significant.

Adolescent

Unfortunately, few sera from adolescents in the general population have been tested and no comparison between subnormals aged 10–19 and normals is possible.

Young adults

As can be seen from Table 1, the percentage of positives did not differ appreciably between defectives in their third and fourth decades of life. They are therefore considered together. As a standard of comparison the best figure available for the general population of these ages is a group of sera from expectant mothers, sera from mothers of stillborn, premature or control normal babies, or cord blood from their babies, and a few sera from other women and some men between 20 and 40 years of age. These sera were tested at the same time as those of the subnormals and at the same laboratory.

Table 3 compares the incidence of positive dye tests of subnormals of this age with that found in these controls. The high proportion of females in the control group is acceptable because as Table 6 shows, at this age there is no significant difference in incidence between the sexes.

The slight excess of positives in the subnormals is not statistically significant. (Difference/standard error = 1·02.)

Table 3. *Dye-test titres of young adults*

Age groups	−ve	+1/8 −1/32	+1/32 −1/128	+1/128	Total	Total +ve	% +ve 1/8
Subnormals aged 20–40	134	29	41	3	207	73	35·2
Controls aged 20–40	76	13	17	2	108	32	29·5

Table 4. *Percentages of subnormals aged* 40–59 *with positive dye test compared with those reported by Beattie in blood donors in rural districts in Lincolnshire*

	1/8 or over	1/32 or over	1/128 or over	1/256 or over	No. tested
Subnormals aged 40–59	52·8	25·9	1·9	0·5	216
Blood donors from rural areas (Beattie, 1958)	49	24	3·6	0·2	505

Older adults

The best standard for comparison available for the older adults is the data published by Beattie (1958) on blood donors in urban and rural areas in Lincolnshire. These sera were taken some years earlier and were tested at a different laboratory. As most blood donors are aged between 40 and 59 years, it is reasonable to compare them with subnormals of this age range, but allowance must be made for differences between laboratories and in time.

Table 4 compares the subnormals in the age range 40–59 years with the figures published by Beattie and shows clearly that, by this age, the incidence in adult defectives approximates to that found in normal rural populations.

Clinical groups

Table 5 compares the incidence of positive dye tests in mongols and epileptics with that in the remaining subnormals.

It is interesting that the incidence of antibodies is as low in the epileptics as in

the mongols since epilepsy is one of the classical signs of congenital toxoplasmosis whereas mongolism, being due to a chromosome anomaly, appears on rational grounds unlikely to be caused by toxoplasma infection. The higher incidence of antibodies in the miscellaneous group than among mongols and epileptics is found in adults, but not in children. This suggests that the excess of dye-test positives in the miscellaneous group may be due to acquired rather than congenital infection.

On comparing the age and sex specific positivity rates (Table 6) it was noted that there was a higher incidence of antibodies in adult males than in females. In those over the age of 40, these differences are greater than one would expect as a result of chance. This might well be due to the increased opportunity to acquire infection of those men who go out to work on farms each day, and as mongols and epileptics tend to be unsuitable for outside employment this would also explain the lower incidence found in these groups.

Table 5. *Dye tests of special groups*

		Mongols			Epileptics			Others		
Age groups		No.	No. +ve	% +ve	No.	No. +ve	% +ve	No.	No. +ve	% +ve
1–19	Male	20	5	27·6	27	6	23·1	78	19	25·2
	Female	9	3		12	3		29	8	
20–39	Male	5	1	25	34	8	25·6	112	46	42
	Female	7	2		9	3		55	24	
40+	Male	5	0	38·5	26	9	39·6	168	110	62·4
	Female	8	5		22	10		81	45	
	Total	54	16	29·6	130	39	30	523	252	48·2

Three patients who were both mongol and epileptic are included in both columns.

Table 6. *Dye-test result by age and sex*

	Female			Male			Combined		
Age groups	No.	No. +1/8	% +1/8	No.	No. +1/8	% +1/8	No.	No. +1/8	% +1/8
1–9	13	4	30·8	34	7	20·6	47	11	23·4
10–19	34	8	23·5	89	22	24·7	123	30	24·4
20–29	37	12	32·5	79	30	38	116	42	36·2
30–39	20	6	30	71	25	35·3	91	31	34·1
40–49	35	12	34·3	81	37	45·7	116	49	42·2
50–59	26	12	46·2	74	53	71·6	100	65	65
60+	18	7	38·9	44	29	66	62	36	58
Total	183	61	33·3	472	203	43	655	264	40·3

Skin-test results

There are no adequate figures available for the incidence of skin-test positivity in the general population of Lincolnshire. The test has not been widely used in England, probably because antigen is not readily commercially available. Messrs

Eli Lilly manufacture antigen in the U.S.A. and were kind enough to supply me with the material used in this survey.

The skin test is simple to perform and lends itself to epidemiological surveys but, as it is unreliable in young children and, even in adults, does not become positive until many months after infection, is of only limited value to the clinician. The delay in appearance of skin sensitivity is so long that the finding of a positive skin test during an illness could almost be taken to exclude the possibility that the illness is due to toxoplasmosis.

Table 7. *Skin test: size of lesion giving best concordance with dye test*

Dye-test titres	Diameter of induration in millimetres										Total	Total concordant	Total discordant
	4 mm.	4	5	6	7	8	9	10	11+	Unsp.*			
−ve	369	5	3	1	5	1	1	3	7	1	396	378 (−)	18
1/8	42	3	—	4	12	7	13	25	28	3	137	88 (+)	49
1/32	33	5	2	5	20	16	13	16	27	6	143	98 (+)	45
1/128	4	1	2	1	3	2	1	1	2	—	17	9 (+)	8
1/256	1	—	—	—	—	—	1	—	1	—	3	2 (+)	1
1/512	1	1	—	—	—	—	—	1	—	1	4	2 (+)	2
1/1024	—	—	—	—	—	—	—	—	1	—	1	1 (+)	—
1/2048	1	—	—	—	—	—	—	—	—	—	1	—	1
1/4096	1	—	—	—	—	—	—	—	—	—	1	—	1
Total	452	15	7	11	40	26	29	46	66	11	703	578	125

* Skin test assessed positive but diameter of induration not recorded.

Although the makers recommend that induration of 10 mm. diameter be taken as the criterion of positivity, it was found that there was better concordance of dye- and skin-test results when 7 mm. was taken as the criterion.

Table 7 shows the relationship between diameter of induration and dye-test titres in 703 patients. It will be seen that, if the 10 mm. standard were adopted, dye and skin tests would be discordant in 29·3% of cases but, if the 7 mm. standard is accepted, they are only discordant in 17·75% of cases.

The 7 mm. standard was adopted in interpreting the results of skin tests in this survey.

As can be seen from Table 8, in 200 (92%) of the 218 persons whose skin tests were positive by this standard the result was confirmed by a positive dye test.

Table 9 is complementary and shows that 378 of the 485 with negative skin tests were also negative to the dye test.

Agreement between skin- and dye-test results, except in children under 10, is good in the women and in the men aged from 20 to 40. It is poor in men over the age of 50. The reason for this is obscure. The discrepancy was noted in several wards in different hospitals which were tested at different times and so is unlikely to be due to faulty technique or deterioration of antigen with storage. Although most of the women were tested before the men, a great many were tested on the same day.

Tables 10 and 11 illustrate this discrepancy.

Table 12 shows the full results of the skin-test survey. As was found in the dye-test survey, the proportion of positives rises with age up to the age of 60 and then declines slightly. The percentage of positives among children is somewhat higher than one would expect, and considerably higher than that found by Burkinshaw, Kirman & Sorsby (1953).

Table 8. *Dye tests of subnormals with positive skin tests*

Age groups	−ve	+1/8 −1/32	+1/32 −1/128	+1/128 −1/256	+1/256 −1/512	+1/512 −1/1024	1/1024	Total	Total +ve	% +ve 1/8
1–9	2	1	1	—	1	—	—	5	3	60
10–19	1	3	7	3	—	1	—	15	14	93·3
20–29	5	12	20	1	—	—	—	38	33	87
30–39	2	12	17	—	—	1	—	32	30	93·8
40–49	4	24	17	2	—	—	1	48	44	92
50–59	2	19	27	2	—	—	—	50	48	96
60+	2	17	9	1	1	—	—	30	28	93
Total	18	88	98	9	2	2	1	218	200	92

Table 9. *Dye tests of subnormals with negative skin tests*

Age groups	−ve	+1/8 −1/32	+1/32 −1/128	+1/128 −1/256	+1/256 −1/512	+1/512 −1/2048	+1/2048 −1/4096	1/4096	Total	Total +ve	% +ve 1/8
1–9	34	2	—	2	1	2	—	1	42	8	19
10–19	92	7	8	3	—	—	1	—	111	19	17·1
20–29	70	5	6	1	—	—	—	—	82	12	14·6
30–39	60	5	3	1	—	—	—	—	69	9	13
40–49	64	6	9	—	—	—	—	—	79	15	19
50–59	33	14	11	—	—	—	—	—	58	25	43
60+	25	10	8	1	—	—	—	—	44	19	43·2
Total	378	49	45	8	1	2	1	1	485	107	22·2

Table 10. *Females: dye test of subnormals with negative skin test*

Age groups	−ve	+1/8 −1/32	+1/32 −1/128	+1/128 −1/256	+1/256 −1/512	+1/512	Total	Total +ve	% +ve 1/8
1–9	9	1	—	—	1	1	12	3	25
10–19	25	1	2	2	—	—	30	5	16·7
20–29	24	2	1	1	—	—	28	4	14·3
30–39	16	2	1	1	—	—	20	4	20
40–49	21	3	2	—	—	—	26	5	19·2
50–59	13	—	—	—	—	—	13	—	—
60+	11	1	—	—	—	—	12	1	8·3
Total	119	10	6	4	1	1	141	22	15·6

Table 11. *Males: dye test of subnormals with negative skin test*

Age groups	−ve	+1/8 −1/32	+1/32 −1/128	+1/128 −1/512	+1/512 −1/2048	+1/2048 −1/4096	1/4096	Total	Total +ve 1/8	% +ve 1/8
1–9	25	1	—	2	1	—	1	30	5	16·7
10–19	67	6	6	1	—	1	—	81	14	17·3
20–29	46	3	5	—	—	—	—	54	8	14·8
30–39	44	3	2	—	—	—	—	49	5	10·2
40–49	43	3	7	—	—	—	—	53	10	18·9
50–59	20	14	11	—	—	—	—	45	25	55·5
60+	14	9	8	1	—	—	—	32	18	56·25
Total	259	39	39	4	1	1	1	344	85	24·7

Table 12. *Skin-test survey of subnormals*

Age groups	Males			Females			Combined		
	Total	Total +ve	% +ve	Total	Total +ve	% +ve	Total	Total +ve	% +ve
1–9	35	4	11·4	17	2	11·8	52	6	11·5
10–19	125	10	8	57	7	12·3	182	17	9·34
20–29	80	25	31·2	65	14	21·5	145	39	26·9
30–39	71	22	31	61	12	19·7	132	34	25·8
40–49	81	28	34·5	74	23	31	155	51	32·9
50–59	74	29	39·2	67	21	31·4	141	50	35·5
60+	45	12	26·7	65	20	30·8	110	32	29·1
Total	511	130	25·4	406	99	24·4	917	229	25

DISCUSSION

In view of serological evidence that toxoplasma infection is highly prevalent in the community, congenital toxoplasmosis is surprisingly seldom diagnosed. This could be accounted for in a number of ways: maternal infection might occur without transmission to the foetus; foetal infection might occur without causing recognizable damage; or foetal damage may occur but, because of lack of the classical features, not be attributed to toxoplasmosis.

The classical syndrome of congenital toxoplasmosis is of choroidoretinitis, cerebral calcifications, mental retardation or neurological damage and hydrocephaly or microcephaly (Sabin, 1942) and, where several of these signs are present, the conditions would probably be diagnosed. For this reason, the incidence of these signs in proven cases, given by Feldman (1958), probably overestimates the frequency with which they occur in combination, and cases exhibiting only a single manifestation may well escape recognition. Thalhammer (1962) produced statistical and serological evidence to support his hypothesis that many children with unexplained congenital cerebral damage may be unrecognized cases of congenital toxoplasmosis. His figures suggest that about 20% of mentally retarded infants lacking other signs of toxoplasmosis may, in fact, be 'oligosymptomatic' cases.

Serological surveys by other workers have so far failed to confirm this opinion. Cook & Derrick (1961) in Australia found little evidence of toxoplasmosis among 342 patients in a mental hospital. Only ten out of 116 juvenile subnormals of unknown aetiology had positive dye tests and they concluded that toxoplasmosis could not have accounted for more than 7% of their cases.

Fleck (1963) found only one out of 114 sera from mental defectives under the age of 10 submitted to his laboratory by various hospitals to be positive to the dye test at a titre of 1/16 and only seven out of 93 sera from a further series of mentally retarded children were positive at a titre of 1/4. For comparison, he took sera from children not suspected of suffering from toxoplasmosis who were admitted to a children's hospital and some children undergoing a poliomyelitis antibody survey as representative of the general population. If these 'controls' were truly representative, and his figures of 16% for the incidence of positive dye test at a titre of

1/4 and 8% at a titre of 1/16 for children under 10 are correct, toxoplasmosis would not appear to be a significant cause of mental subnormality in the area served by his laboratory. It is, however, possible that, by making use of sera from children admitted to hospital, he may have overestimated the incidence in the general population.

This difficulty, in estimating the incidence of antibodies in the general population, is of considerable importance. As the incidence of toxoplasmosis may vary from year to year and from place to place, it is difficult to ensure comparability of cases and controls.

Several of the children included as controls in this survey, although dye-test negative when tested in 1962, had been reported positive to the test when their sera were tested at a different laboratory in 1960. Until the significance of these observations is known, one must view such statistical and serological evidence with some scepticism.

Despite these reservations, the excess of positivity noted in mentally defective children in hospitals in Lincolnshire is of the same order as that noted by Thalhammer in Vienna. Although the possibility that a proportion of the cases may have been due to congenital toxoplasmosis cannot be denied such a conclusion is not justified by the evidence of this investigation. If the excess of dye-test positivity in subnormal children indicated brain damage resulting from congenital toxoplasmosis one would expect that the incidence in subnormals whose condition was known not to be due to toxoplasmosis would be low. Different environments may modify the degree of exposure to risk of acquiring infection, and not only may the risk differ between hospital and home, but the quality of care provided in the home is a factor in determining which defectives are admitted to hospital.

In an attempt to eliminate these factors it was intended to compare within the hospitals a group whose condition was not due to toxoplasmosis and a group exhibiting one of the common symptoms of congenital toxoplasmosis with the rest. Mongols and epileptics were the largest readily identifiable groups suitable for this purpose. It seemed unlikely that toxoplasma infection would disturb the chromosomes of an ovum and so be a cause of mongolism. Although Ira (1960) reported a higher incidence of positive skin test in mothers of mongols than in mothers of normal children this is more probably due to the association between maternal age and mongol birth or to associated environmental factors than to a causal connexion.

Unfortunately the number of mongols in the hospitals visited proved too small for the calculation of reliable age and sex specific rates. Rates for 20-year age groups were calculated however, and these showed that below the age of 20 there was no difference in incidence between mongols, epileptics, and other subnormals. Differences found in the older groups might well be due to the fact that mongols and epileptics tend to be unsuitable for outside employment, whereas many of the other male defectives go out to work on farms.

Similarly, it is possible that environmental factors may be responsible for the high rates found in all groups of subnormal children. As a result of their condition

such children are bound to lead a very different life from that of normal controls even before their admission to hospital. Since the common mode of transmission of toxoplasma infection is not known we cannot know what environmental differences are relevant here, but it does appear that they are in the homes rather than the hospital, for although the child defectives in hospital showed an excess of dye-test positivity this became steadily less with increasing age and duration of residence in hospital, except in adult males, a number of whom worked outside the hospital. Thus, although the highly significant difference between the incidence in defective children and controls alone would suggest that toxoplasmosis might be a common cause of severe subnormality, comparison of groups within the hospital make it appear more likely that, unless toxoplasmosis is also a cause of mongolism, these differences are more probably due to postnatally acquired infections resulting from environmental factors.

Although the skin test proved far less sensitive than did the dye test, the skin-test survey showed the same trends as did the dye-test survey. Some of the discrepancies noted related to patients with high dye-test titres, suggestive of recently acquired infection which had not yet resulted in altered skin sensitivity. Many of the discrepant results, however, were in patients having only low dye-test titres. These are puzzling and suggest that skin-test sensitivity may not always be maintained for as long after infection as is the production of circulating cytoplasm modifying antibody. The fall in incidence of positivity to both tests in the oldest defectives is more probably due to a tendency for both antibody levels and dermal sensitivity to fade below the arbitrary 'threshold' at which we detect them than to higher mortality among those with positive tests. If this is the explanation, the discrepant results would be explicable on the basis of variation in the relative rates at which antibody levels and dermal sensitivity faded in different individuals whose infections had occurred many years previously.

SUMMARY

Of 918 severely subnormal patients in mental deficiency hospitals in Lincolnshire 917 were tested by the toxoplasmin skin tests, forty-nine out of 265 patients were dye tested because of a positive skin test or other indication. All patients in another group of 655 subnormals were dye tested, and comparison of these results with those of groups from the normal population showed that in children there was an excess of about 20% of dye-test positivity among the subnormals—a figure identical with that found by Thalhammer in Vienna.

The excess of positives became progressively less with increasing age and by middle age the incidence was the same in subnormals as among blood donors in the county. It is suggested that this indicates that patients in hospital are exposed to a lower risk of acquiring infection and that the higher incidence among adult males than among adult female defectives is due to the fact that more of them go out to work, many of them on farms. This factor could also explain the lower incidence of positivity in adult mongols and epileptics who are less commonly employable.

Possible explanations of the higher incidence among subnormal than among

control children are discussed. Although it is tempting to assume that it indicates that some may be cases of congenital toxoplasmosis, the lack of gradient between child mongols, child epileptics and other subnormal children does not support this hypothesis. The numbers of mongol and epileptic children were too small to give statistically significant results. It is possible that the home environments of severely subnormal children exposed them to a higher risk of infection than did the homes of the control children and that the observed excess of dye and skin-test positivity may be due to increased incidence of acquired infection before their admission to hospital.

It was found that there was reasonable concordance between the dye test at a titre of 1/8 and skin sensitivity reactions exceeding 7 mm. in diameter, but poor agreement if 10 mm. was taken as the criterion of positivity.

Despite the lesser sensitivity of the skin test, the pattern of the results was the same as that observed in the dye-test survey, and the value of the skin-test for epidemiological surveys was confirmed.

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Histological study of adenovirus type 14 development in cell cultures

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Ever since the first papers concerning the detection of antigenic material in cells by means of antibodies coupled with fluorescein (Coons, Creech, Jones & Berliner, 1942; Coons & Kaplan, 1950) were published, the methods have been modified and improved, either by the use of new fluorochromes and of high-titre antisera, or by the application of more adequate procedures.

Acridine orange (AO) staining as used by Armstrong & Hopper (1959) and Bertalanffy, Masin & Masin (1956) in cell cultures or in smears, is based on the different types of fluorescence in ultraviolet light displayed by nucleic acids after staining.

The development of adenoviruses can be detected in infected cells before the occurrence of cytological changes, by means of fluorescent antibody and acridine orange staining.

So, immunofluorescence was used by Pereira, Allison & Balfour (1959) and Boyer, Denny & Ginsberg (1959), while AO staining was used by Armstrong & Hopper (1959), and Bartolomei Corsi & Harkevitch (1960). In 1964, Mayor applied these methods in an investigation of the changes undergone by ribonucleic acids in cells infected with adenovirus.

The present study is concerned with the changes caused in monkey kidney and KB cells by adenovirus type 14, using these methods.

MATERIALS AND METHODS

Cell cultures

KB cell cultures and secondary Macaccus rhesus kidney cell cultures (MK) on neutral glass cover-slips of 5×20 mm. in 16×160 mm. tubes were used. The culture fluid was Hanks's solution with lactalbumin hydrolysate and 2% or 10% calf serum for MK or KB cultures respectively. After appearance of the monolayer the culture fluid was removed and each cover-slip was inoculated with 0·1 ml. adenovirus type 14 (CPD 50/0·2 ml. = $10^{5.5}$). The same fluid containing 2% calf serum was used for maintenance of the cultures.

The controls consisted of non-inoculated KB or MK cell cultures. The cultures were incubated at 37° C., and samples were collected at definite intervals (6, 8, 12, 24, 36, 48, 72, 96 hr.).

Virus strains

Adenovirus strains grown on KB or MK cells and stored at $-20°$ C. were used. The titres of the stock solutions of virus, computed according to the method of Reed & Muench (1938), are shown in Table 1.

Table 1. *Titres of stock solutions of strains of adenovirus*

Adenovirus type	Titre CPD 50/0·2 ml.
1	$10^{5·5}$
4	$10^{6·5}$
7	$10^{5·0}$
14	$10^{5·5}$
16	$10^{5·3}$

Immune sera

For immunofluorescence we used antisera prepared in rabbits by six intravenous inoculations, at 3 days' intervals, of 1 ml. adenovirus types, 1, 4, 7, 14 or 16. The animals were bled 10 days after the last inoculation. Neutralizing titres average 1/256–1/512.

Conjugation with fluorescein isothiocyanate (ITC)

Immune rabbit sera were purified by precipitation with 60% saturated $(NH_4)_2SO_4$ solution, and the amount of protein obtained was determined by refractometry. For conjugation of the serum with ITC (FLUKA), 0·05 mg./mg. of protein were used, as in the method of Coons & Kaplan (1950), modified by Riggs *et al.* (1958) and by Marshall, Eveland & Smith (1958).

After dialysis for 4–5 days against 0·01 M phosphate buffer, pH 7·2, the conjugated globulin was passed through a Sephadex column. Sodium azide was added to make up a concentration of 0·08%, the sera were distributed in vials and maintained at 4° C.

Before use non-specific fluorescence was removed by absorption of the conjugated serum with human or monkey liver powder (two absorptions). An additional absorption was made with KB or MK cell cultures. The serum was used in a 1/40 dilution.

Fluorescent-antibody staining technique

Infected and control cell cultures were fixed in acetone for 10 min. at room temperature, at various intervals after inoculation. Preparations were air-dried and covered with anti-adenovirus serum conjugated with ITC and diluted 1/40. A number of preparations were covered with a mixture of conjugated anti-adenovirus serum and complement (fresh guinea-pig serum) in equal parts (final dilution of complement 1/20).

The culture was left in contact with the anti-adenovirus serum for 30 min. at 37° C. in the moist chamber. The preparation was carefully washed with phosphate buffer, pH 7·2, for 20 min., and finally rinsed with distilled water for the removal of phosphate crystals. The preparation was mounted in buffered glycerol, pH 7·2, and examined in ultra-violet light, using a monocular Zeiss microscope and Zeiss

L (HBO 50) mercury vapour lamp, with UG_1, 1·5 mm. screening filter and GG_9 protection filter.

Suitable controls were put up, which are described in detail under Results.

AO staining

For the demonstration of nucleic acid in the cell and of the changes resulting from adenovirus infection fluorochrome AO was used in a 1/40,000 aqueous solution.

Infected and control cultures were fixed in 96% ethanol for 1 min. and stained with AO for 40 sec. They were rinsed with tap water for 3 min., mounted in water and examined in ultra-violet light.

By this method ribonucleic acid (RNA) is stained orange-red and deoxyribonucleic acid (DNA) green.

RESULTS

Fluorescent-antibody staining technique

During the first 8 hr. following inoculation cells infected with adenovirus were not specifically stained by anti-adenovirus serum conjugated with ITC. The control culture displayed the same behaviour. In both cases the cytoplasm was weakly fluorescent, while the nucleus appeared as a dark central area (Fig. 1).

Between 8 and 16 hr. fluorescent granules of various sizes, forming conglomerates or rosettes, appeared in the nuclei of some cells. The nucleoli remained dark (Fig. 2).

After 16 to 24 hours the fluorescent material appeared either as lumps, as above, or filled the whole area of the nucleus. The nuclei at this stage were as a rule seen to be hypertrophic. Background fluorescence of the cytoplasm became more intense in certain cells (Fig. 3).

Between 24 and 48 hr. about 80% of the cells were displaying specifically stained nuclear material. The nuclei were large, deformed and contained conglomerates of fluorescent material (Figs. 4, 5). Some of the cells appeared as compact fluorescent masses. In the other cells, the fluorescent mass was placed like two central symmetrical blocks (Fig. 6). The unstained areas corresponding to the nucleoli, as seen in the preceding stage, had disappeared.

At the end of 72 hr. and later, advanced lesions were seen progressively to prevail.

The specificity of fluorescent staining was demonstrated by the negative results with the following controls:

KB and MK cultures infected with adenovirus type 14 + normal rabbit serum conjugated with ITC.

KB and MK cultures not infected with adenovirus + anti-adenovirus type 14 serum conjugated with ITC.

Serum inhibition control: KB and MK cultures infected with adenovirus type 14 + unconjugated homologous immune serum; incubation for 30 min. at 37° C.; addition of type 14 anti-serum conjugated with ITC; incubation for 15 min. at 37° C.

As controls for the detection of possibly existing type specificity, we used:

KB and MK cell cultures infected with adenovirus type 14+type 1, 4, 7 or 16 antiserum conjugated with ITC.

KB and MK cell cultures infected with adenovirus types 1, 4, 7 or 16+type 14 antiserum conjugated with ITC.

In both cases fluorescence was similar to that recorded in the specific reaction of the antigen with homologous type serum.

The addition of complement to the conjugated serum resulted in an increased intensity of fluorescence without any change in non-specific background fluorescence.

No differences in the appearance and course of adenovirus infection were found to exist between KB and MK cells.

AO staining

AO staining afforded the possibility of discriminating between the nucleic acids of the cell. In normal KB or MK cells the cytoplasm displayed a reticulum stained orange-red. The spaces within the reticulum were non-fluorescent. As a rule, the cytoplasm around the nucleus appeared to be condensed. The nucleus displayed a dull green fluorescence and contained varying numbers of nucleoli stained an intense orange-red (Fig. 7).

In infected KB or MK cells the first changes in the staining properties of the cell constituents appeared about 8 hr. after the infection. The first detectable change consisted in a slight increase in size of the nucleus which contained bright yellow-green granules. The nuclei displayed a normal pattern (Fig. 8).

After 24–48 hr. the number of cells with large nuclei had increased. The nuclei contained bright yellow-green lumps (DNA). At the same time orange threads and granules of RNA, or even peripheral or central nuclear areas with processes reaching the cytoplasm, could be seen in some nuclei. In other places the granules of DNA were surrounded by a fine orange zone. Some nuclei appeared to be separated from the rest of the cytoplasm by non-fluorescent perinuclear vacuolar spaces (Fig. 9).

After 48 hr. the whole mass of the nucleus was stained a bright green. No RNA staining was any longer detectable. The nucleoli had disappeared. At this stage a narrow zone of orange cytoplasm was still detectable at the periphery of large nuclei. Similar results were obtained with both cell lines used, i.e. KB and MK.

CONCLUSIONS

By means of the fluorescent antibody method and of acridine orange staining, the course of adenovirus infection in KB and MK cell cultures could be followed. Both methods appear to be specific and sensitive and permit the early detection of infection.

Thus, a latent period of about 8 hr. was found to exist in the multiplication of adenovirus type 14. The first detectable lesions involved the nucleus and probably corresponded to the appearance of a modified DNA.

The development of the adenovirus, which contains DNA, rapidily results in

important histochemical and morphological alterations of the cell. With the methods used these changes become demonstrable during the first 48 hr., i.e. before the appearance of a cytopathic effect.

The presence of a soluble group antigen could be demonstrated by immunofluorescence. The existence of this antigen results from the reaction of various adenovirus types with the same conjugated immune serum, and vice versa, from the reaction of adenovirus type 14 with various anti-adenovirus sera (types 1, 4, 7, 14 or 16) conjugated with ITC.

SUMMARY

By means of the fluorescent antibody method and of acridine orange staining experimental infection with adenovirus type 14 has been investigated in KB and MK cell cultures. Intracellular changes could be shown to precede the appearance of the cytopathic effect.

Thus, progressive stages in the development of adenovirus at this level could be separated both by the demonstration of the specific antigen and by the appearance of an altered DNA.

Both methods have proved to be equally useful for the detection of virus multiplication and of the histochemical changes induced in the cells.

No significant differences have been found to exist with respect to the cell line used.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Normal KB cells. Fluorescent antibody method. Weak cytoplasmic fluorescence. × 500

Fig. 2. KB cells, 16 hr. after infection with adenovirus type 14. Fluorescent antibody method. Small aggregates of viral antigen and granules are present in the nuclei. × 500.

Fig. 3. KB cells, 24 hr. after infection with adenovirus type 14. Fluorescent antibody method. Large aggregates of viral antigen and specific material taking up the whole cell. × 500.

PLATE 2

Fig. 4. KB cells, 48 hr. after infection with adenovirus type 14. Fluorescent antibody method. Large fluorescent aggregates of viral antigen are seen in the nucleus; increased background fluorescence of the cytoplasm. × 500.

Fig. 5. KB cells, 36 hr. after infection with adenovirus type 14. Fluorescent antibody method. In the left part of the figure a cell displaying granules of viral antigen in its nucleus; in the right part of the figure the nucleus of a cell is filled with a strongly fluorescent mass of viral antigen. × 1250.

Fig. 6. KB cells, 72 hr. after infection with adenovirus type 14. Fluorescent antibody method. A cell displaying two intensely fluorescent nuclear masses in a characteristic 'butterfly' pattern. × 500.

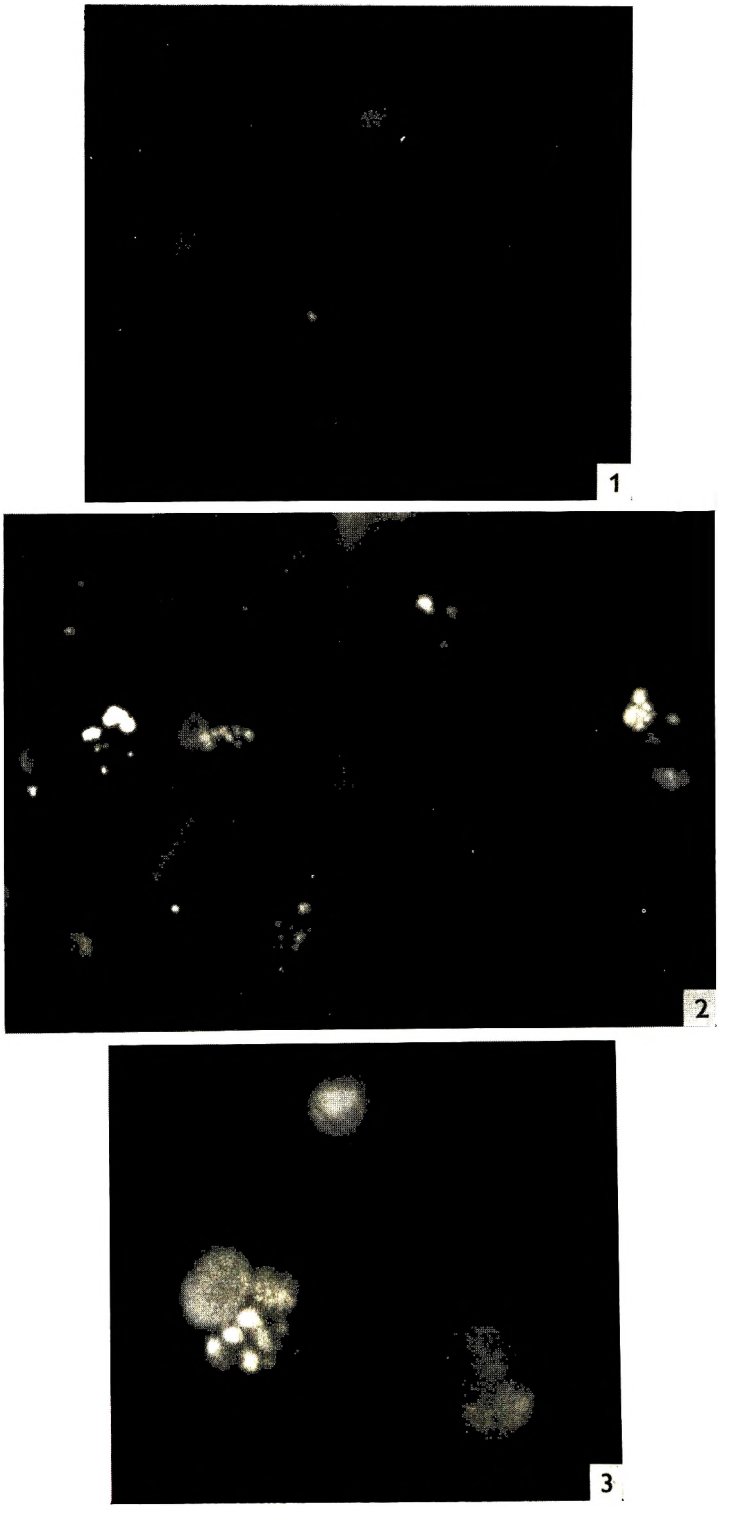
PLATE 3

Fig. 7. Normal KB cells stained with acridine orange. Cytoplasm stains orange-red (RNA), nuclei green (DNA). Nucleoli display an intense orange-red stain.

Fig. 8. KB cells 16 hr. after infection with adenovirus type 14. Acridine orange technique. Nuclei are enlarged. Green inclusions of DNA appeared in the infected nuclei.

Fig. 9. KB cells 24 hr. after infection with adenovirus type 14. Acridine orange technique. Nuclei contain bright yellow-green lumps. Nuclear RNA surrounds the viral inclusion and orange threads reach the cytoplasm. Some nuclei appear to be separated from the rest of the cytoplasm by non-fluorescent perinuclear vacuolar spaces.

Fig. 10. KB cells 48 hr. after infection with adenovirus type 14. Acridin orange technique. Bright yellow-green lumps are placed like two symmetrical blocks.



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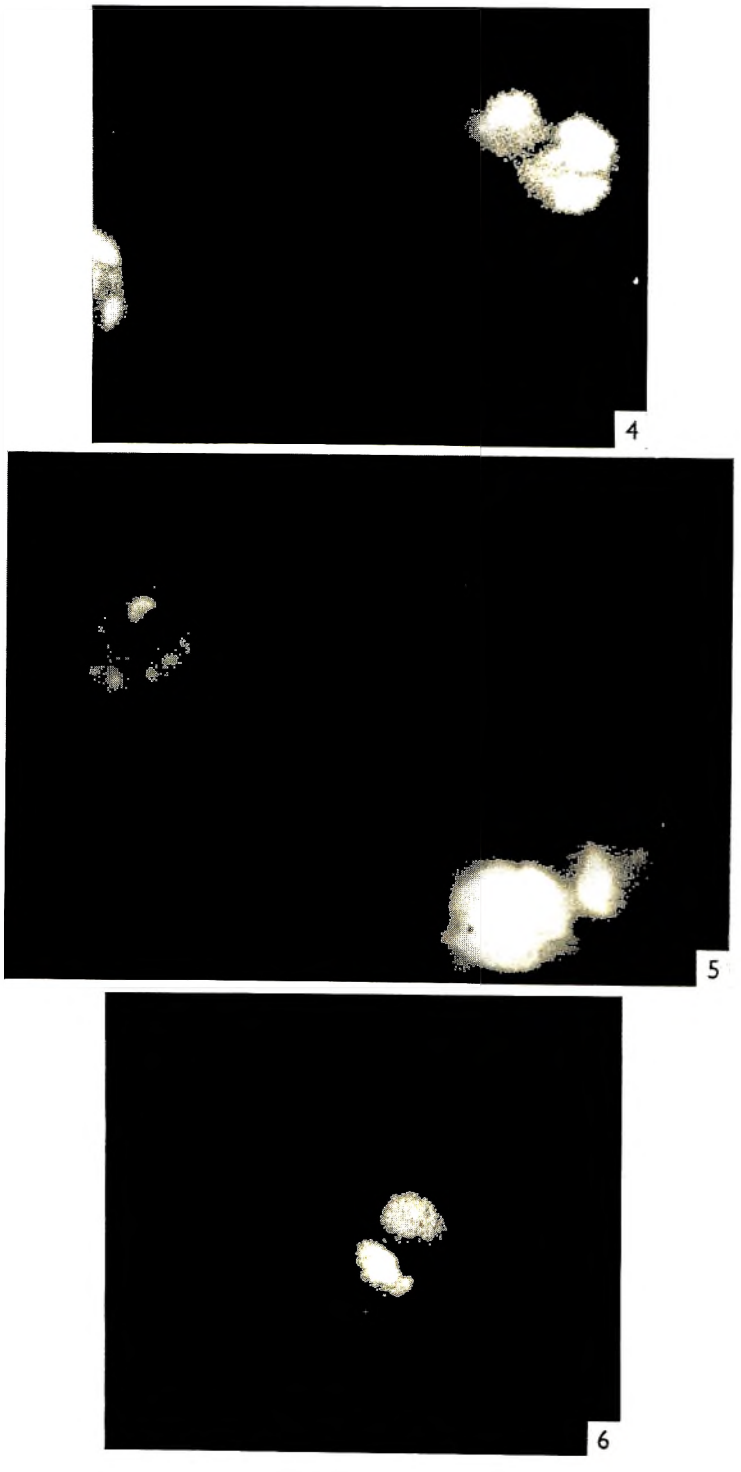
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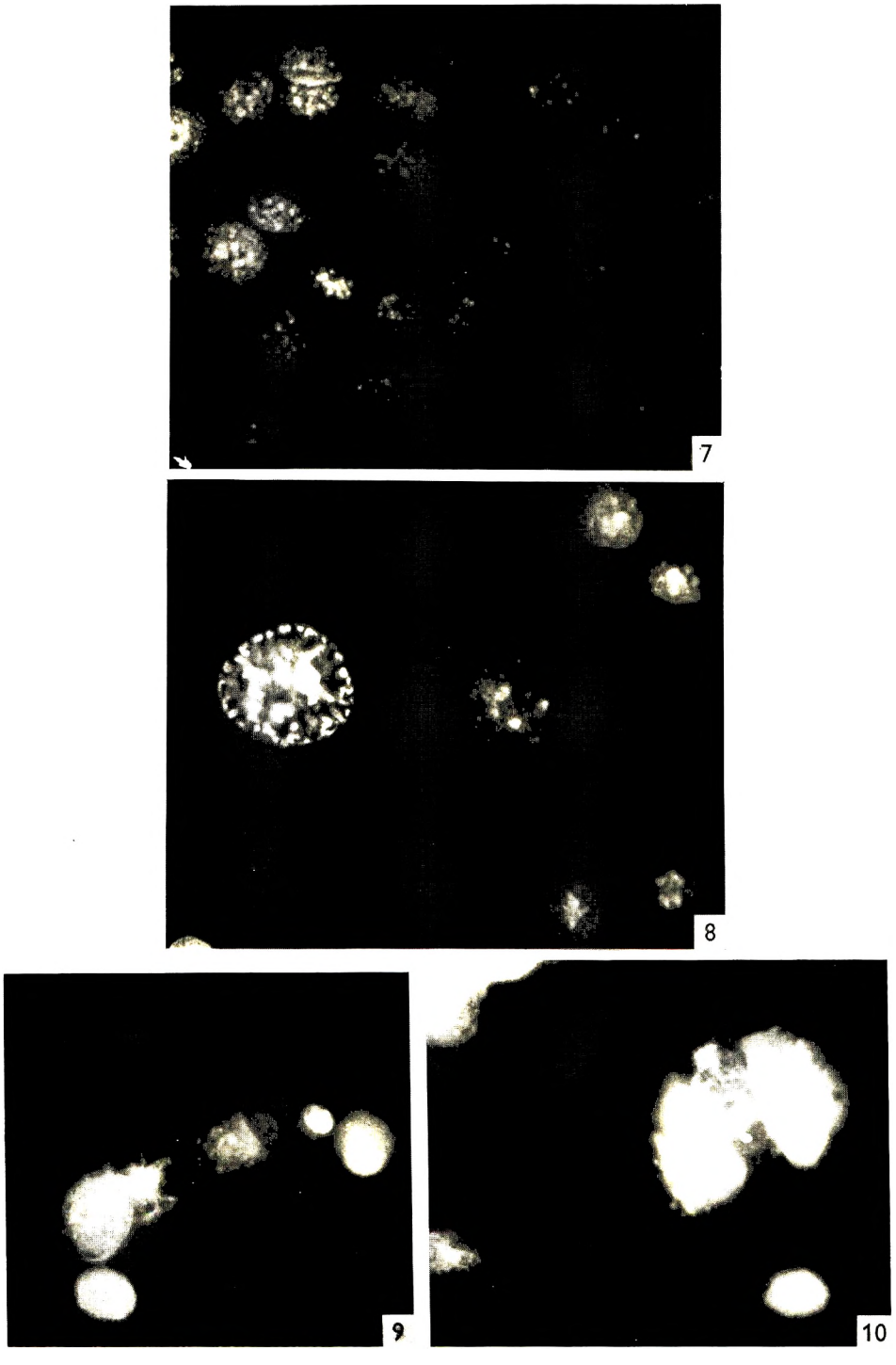
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Staphylococci in noses and streptococci in throats of isolated and semi-isolated Antarctic communities*

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Antarctica is one of the few places left on earth where small groups of men are cut off from physical contact with other communities for long periods of time. Despite recent advances in aerial transport to the south-polar regions, it is still possible to study men who are completely isolated for 6–12 months. This provides unusually simplified conditions for the study of the persistence and exchange of micro-organisms of the respiratory tract (Sladen, 1961).

The only previous Antarctic work on the microbiology of the human upper respiratory tract was done by McLean (1919), the medical officer of the Australasian Antarctic Expedition of 1911–14. He cultured bacteria from the nose and throat of four members of the expedition, two of them for 9 consecutive months, to observe whether there was any diminution or disappearance of organisms. The period extended from the time of leaving Tasmania, December 1911, until the end of the first Antarctic winter. He noted that *Staphylococcus albus* persisted throughout the period, but *Staph. aureus*, though present during the first 3 months, did not appear again during the last 6. McLean identified his staphylococci only by morphological and staining characters. Streptococci also showed a tendency to decrease, but no differentiation was made as to species.

This possibility of a decrease in the numbers of potentially pathogenic organisms in the upper respiratory tract of men living for prolonged periods in Antarctica was investigated in more detail at Hope Bay, Antarctic Peninsula, and at Signy Island, South Orkney Islands. These investigations, carried out during 5 years with the Falkland Islands Dependencies Survey (FIDS), now British Antarctic Survey, have been reported briefly at a meeting of the Pathological Society of Great Britain and Ireland, in July 1952. A more detailed study, which included respiratory viruses, was made with the United States Antarctic Research Program (USARP) at the end of the International Geophysical Year (IGY) on the icebreaker U.S.S. *Staten Island* (Sladen & Goldsmith, 1960). Several hundred bacterial cultures from nose and throat were collected when with FIDS and 2660 cultures during the USARP investigations. Work on respiratory viruses and methods for their study in Antarctic communities will be dealt with elsewhere.

* This paper was presented at the 1st SCAR Symposium on Antarctic Biology, Paris, September 1962, and published in *Biologie Antarctique*, Hermann, Paris (1964). It has been slightly modified, and Table 4 added, to conform with publication in a medical journal.

METHODS AND MATERIALS

The subjects

All were young males with the exception of three at Wilkes Station who were over 40 years. They were picked for Antarctic duties because of their physical fitness. Seven men were studied at Hope Bay in 1948–49 for a period of 9 months, during 7 of which they were completely isolated from the outside world. At Signy Island six men were studied for periods of 1–3 years between 1949 and 1952. Three of these men spent over 2 years in Antarctica with periods of complete isolation of 9 and 8 months each and intervening periods of semi-isolation when the supply ship was visiting the base. Two others spent 9 months, including the first winter, in complete isolation; the sixth man was isolated for 8 months, which included the second winter.

Three groups of men were studied during the USARP investigations in 1958–59:

(i) Fifty-six volunteers from 232 men aboard the icebreaker U.S.S. *Staten Island* during its Antarctic tour. Two of them were civilian medical officers; one of whom (W.S.) had been a member of the Hope Bay and Signy groups. The study of the group began when they left Seattle, U.S.A., in October 1958, and continued during the voyage via New Zealand, McMurdo, Little America, Hallett, Wilkes and Australia, until the return to U.S.A. in April 1959. The icebreaker was in the Antarctic for over 3 months, and at sea for all but 3 weeks of the remaining period. The longest time in port was 6 days at New Plymouth, New Zealand, in November 1958, and this group could therefore be regarded as a semi-isolated community. Thirty-two of these men were finally swabbed at Point Barrow, Alaska, nearly 10 months after the first swabs were taken.

(ii) Twenty-five men who had been completely isolated at Wilkes for 12 months were studied intensively ashore and aboard *Staten Island* during the first month after the arrival of the ship. During this period an average of nine swabs was taken from the nose and ten from the throat of each man, the first samples being obtained on 24 January 1959 before contact with incoming personnel from the ship.

(iii) Sixteen men from Hallett were examined after 9 months of complete isolation and then a period of semi-isolation during the summer. It was not possible to collect more than one or two swabs from these men before they left the Antarctic.

Collection of material and culture methods

All nose swabs were collected by rotating a sterile cotton-tipped applicator in both anterior nares; throat swabs, by stroking firmly both sides of the fauces in the tonsillar region. Swabs were discarded after inoculation on to the culture or holding media.

Cultures from the nose. The FIDS cultures were made under a variety of conditions, often quite primitive. Control swabs taken aboard the expedition ship before it sailed from England, on both occasions December 1947 and October 1949, were inoculated a few hours later on blood agar plates at the Middlesex Hospital, London. All organisms isolated were dry-frozen by the Rayner (1943) method, for later comparison with the Antarctic material. Direct primary cultures on Loeffler

slopes taken during the voyage south in 1948 were incubated in the ship's engine room. Most of the swabs collected in the Antarctic were inoculated immediately on to slopes of Loeffler medium in small screw-capped vials, incubated at 37° C. in a kerosene-heated incubator for about 24 hr. and stored at 4° C., or lower, as direct primary cultures. When time and conditions permitted, swabs were also streaked directly on to 10 % human blood agar plates, subcultured on to Loeffler and stored in the same way. The biology laboratories at Hope Bay and Signy Island needed considerable reconstruction and insulation before they could be used for microbiology. It was necessary to work at night to prevent contamination from other activities, such as cooking, in adjacent rooms. On sledge trips during the Antarctic winter, the culture media had to be thawed before inoculation. A primus cooking stove was used instead of a conventional bunsen burner during inoculation. The screw-capped vials were either handed back to the donors for incubating overnight in their sleeping bags, or suspended for several hours at the peak of the tent, where heat from the primus stove raised the temperature to approximately 37° C. When the expedition returned to England, all the cultures were revived by the addition of 1 ml. of 10 % blood-broth to each vial, incubated for 24 hr., and plated on to blood agar.

The USARP investigations were conducted in a small laboratory 10 × 8 ft. on the main deck of the U.S.S. *Staten Island*. It was not possible to make blood agar plates under these conditions, so, as in much of the FIDS work, each nasal swab was inoculated as a direct primary culture on to a Loeffler slope in a $\frac{1}{4}$ oz. screw-capped vial. As an additional precaution, the same swab was afterwards inoculated on to a mannitol salt agar (Baltimore Biological Laboratories) slope, also in a screw-capped vial. The Loeffler slopes were incubated for 12–18 hr. and the mannitol salt agar for 24 hr., at 37° C., and then stored at 4° C. until the ice-breaker returned to U.S.A. Staphylococci were later isolated from these cultures on blood agar plates in the Clinical Bacteriology Laboratory at Johns Hopkins Hospital.

All staphylococci isolated in pure culture were tested for coagulase properties by the method of Fisk (1940) using pooled human plasma. All staphylococci which proved coagulase positive will be called *Staph. aureus*, those that were coagulase negative, *Staph. albus*. The *Staph. aureus* were bacteriophage typed; those from FIDS men by Dr R. E. O. Williams at the Staphylococcal Reference Laboratory, Colindale, London (Williams & Rippon, 1952), and those from USARP men at Johns Hopkins Hospital with routine test dilution (RTD) using methods recommended by Blair & Carr (1953). Antibiotic-sensitivity tests were done by the disk method (Baltimore Biological Laboratories) and are reported in this paper for penicillin (2 units) only.

Cultures from the throat. All throat cultures collected and recorded at Hope Bay were lost in a fire which destroyed the hut in November 1949 (Fuchs, 1951). At Signy Island, streptococci were isolated either by plating a throat swab directly on to blood agar or by inoculating a Loeffler slope and incubating, storing and reviving in the same way as described for the staphylococci. In the USARP investigations two throat swabs were collected simultaneously at each examination. One was mixed in virus-holding medium for a separate inquiry; the other

in 5 ml. of streptococcal holding medium of trypticase broth containing 15% of glycerine in screw-capped vials. These samples were frozen within 30 min. to minus 60° C. and held in this state until worked on in the following manner at the Johns Hopkins laboratory. The contents of the vials were allowed to thaw at room temperature for about 30 min. and then shaken thoroughly. Two plates (deep pour sheep's blood agar plate and human blood agar streak plate) were then inoculated immediately with one loopful of contents for each plate and the vials promptly refrozen to minus 60° C. Techniques recommended by Schaub *et al.* (1958) for the isolation of β-haemolytic streptococci were used. All pure subcultures were held on Loeffler slopes and sent to Dr Roger Cole, National Institutes of Health, Bethesda, for grouping.

RESULTS

Nasal staphylococci

The staphylococci survived extremely well on the Loeffler slopes no matter how they were collected. *Staph. aureus*, if present, often overgrew other organisms, thus leaving a pure growth. Table 1 summarizes the results of the first year of study

Table 1. *Summary of nasal staphylococci from seven men at Hope Bay, before and during complete isolation in Antarctica*

		Staph. aureus carriers			*Staph. albus* carrier	Insufficient data		
Date	Place and occasion	W. S.	B. J.	S. M.	M. G.	O. B.	J. O.	F. E.
Dec. 1947	England	A 1	A 3	a	ng	—	a	—
Jan. and Feb. 1948	Aboard ship at sea	A 1	A 3a	A 4	ng	—	—	a
Complete isolation*								
April	Hope Bay, before sledging	a	A 3	A 4	a	ng	a	A 2
June	Hope Bay, after sledging	A 2	A 3	A 4	a	A 2a	a	a
July	Hope Bay, before sledging	A 3	A 3	—	ng	a	—	ng
Aug.	Hope Bay, after sledging	A 2	A 3	ng	a	ng	A 3	ng
Sept.	During 4th sledge journey	A 2	—	—	a	—	—	—
Sept. 1948	Hope Bay, after sledging	A 2	A 3a	ng	a	a	—	—

a = *Staph. albus* isolated; ng = no staphylococci isolated from original culture; — = no swab taken.

A = *Staph. aureus* isolated. Phage patterns: A 1 = 3A/3B/3C/51; A 2 = 53, or 42B/53/54; A 3 = 6, or 6/47, or 47/53; A 4 = 29, or 29/79.

* Complete isolation was from 1 March to end September 1948 (7 months).

at Hope Bay. Two men (W. S. and B. J.) and probably a third (S. M.) carried *Staph. aureus*, and one man (M.G.) *Staph. albus*, consistently throughout 6–9 months. There are insufficient data from the other three members of the party, but they carried both *Staph. aureus* and *Staph. albus*, at one time or other. *Staph. aureus* persisted in at least two individuals (W. S. and B. J.) for at least 7 months of Antarctic isolation at Hope Bay. These observations differ from those of McLean (1919), who was unable to isolate *Staph. aureus* from noses after the first 3 months of isolation.

Each *Staph. aureus* carrier kept the same phage type in his nose for a long period of time. This shows well in B. J., who held his for 9 months, and fairly well in the other two subjects. W. S. carried phage type 3A/3B/3C/51 before leaving England in December 1947 and kept this during the voyage out. Sometime between January and June 1948 the phage type changed to a completely different one. W. S.'s first sample of phage type 3A/3B/3C/51, collected in December 1947, was dried independently of the second sample collected at sea in January 1948.

Table 2. *Summary of nasal staphylococci from six men at Signy Island, South Orkney Islands, before, during and after complete isolation in Antarctica*

			Staph. aureus and *Staph. albus* carriers					
Date		Place and occasion	W. S.	J. C.	R. W.	E. S.	J. B.	D. D.
Oct. 1949		Before leaving England	a	A5a	ng	A7	.	–
Sept. 1950	Complete isolation	During sledge journey	A2	A5a	A6	–	.	–
Sept.		Signy, after sledging	A2	A5	ng	A7	.	A2a
Nov.		Signy, before relief ship	A2a	A5 & A2	A6	A7a	.	a
Dec.	Semi-isolation	Signy, after relief ship	–	A5	–	A7	A8	–
Jan. 1951		Signy, before 2nd ship	A2	A5a	ng	–	A8a	–
April		Signy, during 3rd ship	A2	A5	A6	–	A8	–
Dec.		England	A2*	–	–	–	–	–
Mar. 1952		England	A2	–	–	–	–	–
Jan.–May		Falkland Islands	–	A5†	a†	a†	A9‡	A10‡
May–July		England	A2	A5	A6a	–	–	A10
Sept. 1952		England	A2	A5a	a	–	–	–

a = *Staph. albus* isolated; ng = no staph. isolated from original culture; – = no swab taken.
A = *Staph. aureus* isolated. Phage patterns; A2 = 53; A5 = 3C or 55 or 3C/55; A6 = 3A; A7 = 29 or 29/75 or 29/47/75; A8 = 42B; A9 = 42E/47; A10 = 52.
Men returned to civilization after: * = 16 months in Antarctica with 9 months complete isolation; † = 27 months in Antarctica with two winter periods of 9 and 8 months each of complete isolation; ‡ = 12 months in Antarctica with 8 or 9 months complete isolation.

The second year of work at Signy Island is summarized in Table 2. All six men were *Staph. aureus* and *Staph. albus* carriers. J. C. carried his *Staph. aureus* phage type consistently throughout 12 months of complete and semi-isolation in Antarctica and still carried it 4 months after returning to Britain after completing a second year in the Antarctic. R. W. was nearly as consistent a carrier as J. C. The staphylococci from W. S.'s nose, continuing from Table 1, are followed for a total of nearly 5 years. The only change in phage type from June 1948 to September 1952 was recorded in July 1948 when he was carrying the same type (6/47) as found in B. J.'s nose. However, on this date W. S.'s usual type (53) was isolated from his throat. It can therefore be concluded with reasonable certainty that W. S. carried one phage type of *Staph. aureus* consistently for over 4 years.

Staphylococci were grown from 773 swabs collected from volunteers aboard U.S.S. *Staten Island*, *Staph. aureus* being grown from 415 swabs and *Staph. albus* from 581. All fifty-six volunteers (100 %) carried *Staph. albus* at one time or other and over half of them persistently throughout the voyage. From the twenty-five

Wilkes men returning from 12 months complete isolation, staphylococci were grown from 223 swabs, 200 of which grew *Staph. albus* and only fifty-seven *Staph. aureus*. Like the *Staten Island* community, the Wilkes men showed a 100 % carrier rate of *Staph. albus*, but more of them (76 %) were persistent carriers.

Table 3. Staphylococcus aureus *from noses of men on U.S.S.* Staten Island *and at two Antarctic stations after isolation; compared with data from medical students*

	Total men swabbed	Total swabs growing *Staph. aureus* and/or *albus*	Av. no. swabs per person	Av. period (weeks) followed	*Staph. aureus* carriers			Total swabs growing *Staph. aureus*	Total *Staph. aureus* penicillin resistant
					Pers.*	Int.* and occas.	Total carriers		
U.S.S. *Staten Island* volunteers	56	773	14	33	8 (14 %)	39 (70 %)	47 (84 %)	415	106 (26 %)
Wilkes	25	223	9	4	4 (16 %)	5 (20 %)	9 (36 %)	57	2 (4 %)
Hallett	16	27	2	1	Not followed for long enough		6 (38 %)	8	1
Medical students†	520	—	—	37	(24 %)	(57 %)	(81 %)	503	(24 %)

* Persistent carriers: number of men from whom *Staph. aureus* was isolated from at least 90 % of the swabs. Occasional carriers: number of men from whom *Staph. aureus* was isolated from less than 10 % of their swabs. Intermittent carriers: between persistent and occasional carriers.

† From Gould & McKillop (1954*a*, *b*).

Staph. aureus carriage is summarized in Table 3. In accordance with Gould & McKillop (1954*a*), these carriers have been divided into three types.

(i) *Occasional carriers* from whom *Staph. aureus* was isolated from less than 10 % of the swabs in any one individual.

(ii) *Persistent carriers* from whom the organism was isolated from 90 % or more of the swabs in any one individual.

(iii) *Intermittent carriers* showed a carrier rate of between 10 and 90 %. Gould & McKillop stressed that persistent and intermittent carriers usually held the same phage type, whereas the occasional carriers did not. Our experience (Sladen *et al.* in preparation) does not fully support this, so for the present it is best to describe the carrier rates on a percentage basis only.

There are some interesting contrasts between the ship's population and the Wilkes men. Forty-seven (84 %) out of fifty-six *Staten Island* men carried *Staph. aureus* on one occasion or more throughout the voyage, whereas only 36 % of the Wilkes men carried them during the month of intensive swabbing after 12 months of complete isolation. Persistent carriers were about the same in both communities (14 % for *Staten Island* and 16 % for Wilkes), but the combined intermittent and occasional carriers were much reduced in the Wilkes group (20 %, in contrast to 70 % aboard *Staten Island*). Sixteen men from Hallett, a group of men who had been mixing a little with a few personnel from relief planes during summer

but who were essentially still isolated after 9 months of complete isolation, had but six individuals (38 %) from whom *Staph. aureus* was cultured, much the same total carrier rate as in the Wilkes men.

Bacteriophage typing showed that over half of the persistent and intermittent carriers aboard the *Staten Island* (22 out of 40), and all six of the Wilkes men, were consistently carrying their own strain within the broad classification of the groups. The typing is now being repeated with concentrated bacteriophage (1000 × RTD).

From a total of 415 swabs which grew *Staph. aureus* in the *Staten Island* volunteers there were isolated 106 (26 %) resistant to penicillin, whereas only two (4 %) out of fifty-seven isolated from the Wilkes men were resistant. These two strains from Wilkes were from two individuals who were occasional carriers, so from none of the four persistent carriers could resistant *Staph. aureus* be isolated. Five out of the eight persistent carriers aboard *Staten Island* carried resistant strains, though only two of these carried them consistently. One of these two men (W. S.) who in 1947–52 had been a penicillin-sensitive *Staph. aureus* carrier was now a persistent resistant carrier. More will be written about his nose, which has been studied for 15 years.

Table 4. *Haemolytic streptococci from throats of men on U.S.S.* Staten Island *and at Wilkes Station*

	Total men swabbed	Swabs: Total	Swabs: Av. per man	β-Haemolytic streptococcal carriers*: All groups	β-Haemolytic streptococcal carriers*: Group A	β-Haemolytic streptococci isolated: All groups	β-Haemolytic streptococci isolated: Group A
Staten Island	56	681	12	20 (36 %)	8 (14 %)	129 (19 %)	47 (7 %)
Wilkes	25	251	10	4 (16 %)	1 (4 %)	5 (2 %)	1 (0·4 %)

* One or more swabs positive per person.

Throat streptococci

McLean (1919) did not differentiate between the α-haemolytic and β-haemolytic streptococci. He reported that streptococci diminished during Antarctic isolation.

α-Haemolytic streptococci. FIDS and USARP collections showed that these streptococci could be cultured from the throats of all personnel before, during and after Antarctic isolation and semi-isolation.

β-Haemolytic streptococci. No attempt was made to culture these organisms during the FIDS investigations. However, good results were obtained from the frozen material collected with USARP (Table 4). Of fifty-six *Staten Island* volunteers there were twenty (36 %) from whom β-haemolytic streptococci were grown on one occasion or more, five of these individuals being consistent carriers (75 % or more of the swabs were positive) throughout the voyage. Group A streptococci were grown from eight (14 %) men and two of these were consistent carriers.

From 681 swabs (averaging 12 swabs per man) collected, there were grown 129 (19%) β-haemolytic streptococci, of which 47 (7%) were group A.

There was a marked difference in the Wilkes men, from whom 251 swabs were taken (averaging 10 per man). Only five (2%) of the swabs grew β-haemolytic streptococci, and only 1 (0·4% of the 251 swabs) grew a group A organism. These five positive swabs were from 4 (16%) of the 25 men. No β-haemolytic streptococci were found on 24 January, the day the men broke their 12 months of Antarctic isolation. Two were grown from swabs taken on 27 January (one group C and another of an unidentified group), one from swabs taken on 8 February (group C) and two from swabs taken on 16 February (groups A and B). There was also an appreciable difference between the number of colonies grown from the four Wilkes men and from the twenty *Staten Island* volunteers. The two consistent carriers of group A on the ship gave colony counts of usually between 10 and 100, occasionally over 100, while the Wilkes cultures, collected, stored and revived in the same way, grew single colonies per plate, or at most seven. Moreover, three of the five positive Wilkes cultures were grown from swabs collected 2 weeks or more after isolation had been broken, and at a time when the men were freely mixing with the men of the icebreaker. Thus, from this small Antarctic Station there seems to be good evidence of a greatly reduced carrier rate, probably amounting to total absence of β-haemolytic streptococci, and certainly of group A streptococci, in men returning after complete isolation from the outside world.

DISCUSSION

Nasal staphylococci

The FIDS results demonstrate, contrary to McLean's (1919) findings, that the potential pathogen *Staph. aureus* can be carried in the anterior nares throughout Antarctic isolation and, in certain individuals, with remarkable persistency. Though the number of swabs collected at Hope Bay and Signy was small, we can be reasonably certain that three (W. S., B. J. and J. C.) out of twelve men were persistent carriers. FIDS men were isolated as a community, but were also living in extremely close contact with each other. A persistent *Staph. aureus* carrier (W. S.) shared a tent with a persistent *Staph. albus* carrier (M. G.) at Hope Bay for 1 month of sledging. W. S. also shared tents with *Staph. aureus* carriers J. C. and R. W. during Signy sledge journeys, yet there was no exchange of strains among them. When at base there were four examples of W. S.'s strain and one of B. J.'s strain being picked up in the anterior nares of other men at Hope Bay and Signy, but they were not held more than once. It is interesting to note that in November 1950 J. C. held W. S.'s strain (76/77) as well as his own (3C). However, 76/77 was subcultured from the original blood agar plate as a single colony, whereas 3C was picked from a different-looking colony growing profusely on the same plate. We can conclude, therefore, that the true inhabitant of J. C.'s nose was a *Staph. aureus* of phage type 3C which he held consistently during $2\frac{1}{4}$ years in Antarctica and for another 9 months back in civilization. This would fit Gould and McKillop's (1954*a*) definition of a persistent carrier, but W. S. would not. W. S.

changed from a group I phage type (3A/3B/3C/51) to a group III phage type (53) and then held the latter consistently throughout two winters of complete isolation and for varying periods back in civilization, for a total of nearly 4 years. Six years later, when aboard U.S.S. *Staten Island*, he changed his phage type at least once (Sladen, in preparation). Yet all of the sixteen swabs taken during this voyage grew profuse colonies of typical golden-yellow coagulase-positive *Staph. aureus*. It seems that, the longer an individual is followed, the more chance there is of recording a change in the phage patterns of his commensal staphylococci. I therefore believe that a persistent *Staph. aureus* carrier should be defined as one who carries this potential pathogen in his anterior nares for 90 % or more of the swabs, regardless of occasional changes of phage type.

Table 3 points to some interesting similarities and contrasts between the *Staten Island* and Antarctic communities when compared with medical students studied by Gould & McKillop (1954*a*). The total carrier rate was about the same for the *Staten Island* men as it was for the Scottish medical students (84 and 81 % respectively) but it was appreciably lower (36 and 38 %) in the Wilkes and Hallett men after Antarctic isolation. The period for which these different groups were followed varied, and it could be argued that 4 weeks with an average of nine swabs per man for Wilkes Station was hardly sufficient to obtain a true comparison with the *Staten Island* and medical student groups. However, data from twenty-two staff members of an English hospital whose noses were swabbed on 8 consecutive weeks gave a carrier rate of 82 % (Williams, 1946), and 104 Australian nurses followed for 16 weeks, but with only four swabs taken per person, also gave an equally high total carrier rate of 85 % (Rountree & Barbour, 1951). Thus the total carrier rate of the volunteers aboard the icebreaker differs little from that of other urban groups followed, whereas there appears to be a marked decrease among men who have been isolated for a long period in Antarctica.

This low carrier rate is of great interest medically, because recent surveys of adults in hospitals and clinics have shown opposite trends. For example, within 5 weeks of trainee nurses entering hospital wards, the nasal carrier rate, based on two examinations per person, rose from 53 to 71 %, and the rate of penicillin resistance from 4 to 32 % (Rountree & Barbour, 1951). Williams *et al.* (1959) give similar figures from patients during their stay in surgical wards. Of 602 patients swabbed within a few days of admission, 38 % carried *Staph. aureus* and 13 % carried penicillin-resistant strains. Of twenty-five swabbed after 8 weeks in the wards, 68 % were carriers and 52 % had resistant strains.

The FIDS results, based on small numbers followed for several years, provide strong evidence that persistent carriers of *Staph. aureus* remained so, before, during and after Antarctic isolation. Unfortunately, it was not possible to collect swabs from the Wilkes and Hallett men before and during their isolation. However, the persistent carrier rate remained much the same in these men after isolation as it did in the *Staten Island* men and the Scottish medical students (Table 3). It thus appears that the reduction in total carrier rate was due to a decrease in the intermittent and occasional carriers, and not in the persistent carriers.

Throat streptococci

During the voyage of U.S.S. *Staten Island* there were two distinct epidemics of upper respiratory infection (URI), and other spasmodic occurrences. None of these could be clinically attributed to acute streptococcal infection, nor could bacteriological findings from the eight group A carriers be correlated. In fact four of these men had no URI's at all, the others having one typical 'common cold' each, two with initial mild sore throats, two without.

The carrier rate of β-haemolytic streptococci and, when available, of the group A streptococci was much the same among the *Staten Island* volunteers as found in adults by other workers (e.g. Straker, Hill & Lovell, 1939; Zanen, Gaynor & van Toorn, 1959; Myers & Koshy, 1961). However, during the past three decades there have been no studies on isolated or semi-isolated communities. Working in the semi-isolated tropical Virgin Islands, Milam & Smillie (1931) reported 13% β-haemolytic streptococci and 5% group A streptococci from a total of 694 cultures, while Paul & Freese (1933) found that only 0·3% of the organisms isolated from a Spitzbergen community of Norwegians were β-haemolytic streptococci. Unfortunately, these and other papers published during this period give no precise data for the carriage of β-haemolytic streptococci throughout and immediately after isolation, so there is nothing to compare with the Wilkes results.

During a 5-year study of school children, Quinn & Martin (1961) showed that the carrier rates and serological types of group A streptococci changed from school to school and from year to year. They suggest that these changes involve complex mechanisms including type-specific immunity of the host, meteorological conditions and interactions within the community. Antarctic communities are characteristically free from acute URI's during their periods of complete isolation. The absence of group A streptococci in the throats of men returning from 12 months isolation suggests that these organisms disappear from the community despite the presence of susceptible hosts. Men cannot be reinfected until they return to civilization and mix with carriers. The same is possibly true for the respiratory virus agents.

What community size can maintain these potentially pathogenic agents? What time must elapse in a small isolated community before the agents disappear from the upper respiratory tract? Does the polar climate have any effect on the disappearance of β-haemolytic streptococci and the reduction of *Staph. aureus* carriers, or is this essentially the result of the isolation of a small community? How do the organisms regain a footing in the nose and throat? For answers to these and other epidemiological questions it will be necessary to study groups before, during and after isolation in several places, and to have better facilities for microbiological research.

SUMMARY

The Antarctic provides unusually simplified conditions for the study of the persistence and exchange of micro-organisms of the upper respiratory tract. The work reported here was done while the author was in the Falkland Islands Dependencies Survey and the United States Antarctic Research Program.

Staph. aureus and *Staph. albus* persisted in the noses, and α-haemolytic streptococci in the throats of men throughout long periods of isolation and semi-isolation in Antarctica.

On the whole, men kept their own strains (phage types) of *Staph. aureus* despite living in very close contact with each other.

Persistent carriers of *Staph. aureus* (90 % or more positive swabs per individual) continued to carry this organism for as long as 2 years in Antarctica. Data from men at Wilkes and Hallett IGY Stations indicated that there was a decrease in the intermittent and occasional carrier rates, resulting in a much lower total carrier rate after 12 months Antarctic isolation.

Evidence is presented to suggest that β-haemolytic streptococci had disappeared from throats after 12 months of isolation. It is thought that the absence of upper respiratory infections in these communities is due to absence of the bacterial or viral agents.

There is an urgent need for further work on the carriage of micro-organisms in the present unique epidemiological conditions of the Antarctic, and for better laboratory facilities there.

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The immunization of mice, calves and pigs against *Salmonella dublin* and *Salmonella cholerae-suis* infections

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Following the development of live vaccines that protected chickens against *Salmonella gallinarum* infection (Smith, 1956*a*), attention has been turned to finding methods of immunizing calves against *S. dublin* infection and pigs against *S. cholerae-suis* infection, the principal salmonella infections of these two species of animals. Chickens were used in all the *S. gallinarum* studies. Those on *S. dublin* and *S. cholerae-suis* were rendered more difficult by the fact that it was not economically possible to use calves and pigs for the bulk of the experiments. These had to be performed on mice, part of the original plan being to find stable live vaccines which were avirulent for these animals but which protected them against oral infection with a fully virulent strain. Any such vaccines would finally be tested in calves or pigs. The limitations of this approach were realized when the vaccine most suitable in protecting mice against *S. dublin* infection, no. 17A, was found to be non-immunogenic in calves, whereas other vaccines which had been rejected initially because they possessed a considerable degree of virulence in mice were subsequently found to be both avirulent and immunogenic in calves.

This paper records the results of experiments to determine the immunogenicity of dead vaccines and antisera in mice, and the results obtained with live vaccines, experimentally with mice and both experimentally and against natural infections in the field with calves and pigs.

MATERIALS AND METHODS

Animals

Mice. Male White Swiss mice were used. They were fed on diet 41B (Oxoid) *ad lib.*

Pigs. Landrace × Large White pigs of both sexes were employed. They were kept under ordinary conditions of management and fed on a proprietary pig meal *ad lib.*

Calves. Unless otherwise stated, male Ayrshire calves were used. They had been weaned at approximately 1 week old and then fed solely on a proprietary liquid milk substitute twice daily. The ages of the mice, pigs and calves when their immunity to salmonella infection was challenged were 8, 11 and 6 weeks respectively.

Bacterial cultures

All vaccines, challenge inocula and agglutination suspensions were derived from smooth fully virulent strains of salmonellae, one strain each of *S. dublin*, no. 188, *S. cholerae-suis* var. *kunzendorf*, no. 195, *S. typhi-murium*, *S. gallinarum*, no. 9, and *S. pullorum* being used throughout. The smooth (S) and rough (R) nature of cultures were assessed by the acriflavine slide test (Braun & Bonestell, 1947).

Salmonella dublin *antisera*

Antisera were prepared in rabbits and calves. Those against dead bacteria were prepared by the multiple intravenous injection of a fully motile nutrient broth culture of strain 188 that had been killed at 56° C. for $\frac{1}{2}$ hr. Those against live bacteria were prepared by the multiple oral and subcutaneous administration of a 24 hr. nutrient broth culture of 188 to animals that had recovered from experimental infection. All sera were sterilized by filtration through a Seitz E.K. pad before use, and were given by subcutaneous injection 2 hr. before challenge.

Vaccines

The exact methods of preparing both live and dead vaccines will be referred to later; those for the 9S and 9R vaccines of *S. gallinarum* have been described previously (Smith, 1956*a*). All live vaccines were employed as single subcutaneous injections of 24 hr. nutrient broth cultures containing approximately 50×10^7 viable bacteria per ml. 3 weeks before challenge except in the experiments shown in Tables 5 and 8; 0·1 ml. were given to mice and 5·0 ml. to calves and pigs. Dead vaccines were employed as two subcutaneous injections of 0·1 ml. for mice and 5·0 ml. for pigs 3 and 2 weeks before challenge. Before vaccination the body temperature, appetite and general appearance of the calves and pigs were recorded and their faeces examined to confirm that they were healthy and free from salmonella infection. These examinations were continued at frequent intervals for 14 days after vaccination. Any animals that died after vaccination were examined for pathological lesions and their livers cultured bacteriologically.

Method of challenge

After an overnight fast, animals were given, by mouth, an aqueous suspension of an 18 hr. nutrient agar culture of the challenge strain to which was added a powder consisting of powdered chalk, 40 %; colloidal kaolin, 43 %; magnesium trisilicate, 17 %. Each calf or pig was given 15 ml. of suspension containing 10^{10} viable organisms and 6 g. of the powder and each mouse 0·05 ml. of suspension containing 5×10^8 viable organisms and 0·03 g. of powder. To facilitate administration the mice were anaesthetized with ether and, when they were recovering, the infecting material was dropped into the mouth by means of a fine pipette.

The examinations on the calves and pigs performed after vaccination were continued after challenge. All animals, including mice, that died were examined for lesions and the liver and, frequently, other organs examined bacteriologically to confirm that they had died from the particular salmonella serotype with which they

had been challenged. The experiments on mice were terminated 19 days after challenge when deaths were uncommon, those on calves at 21 days when the survivors had recovered and those on pigs at 14 days when the survivors had either recovered almost completely or were so emaciated that destruction was necessary on humane grounds.

The examination of faeces and organs for salmonellae

Rectal swabs, taken so as to include a liberal portion of faeces, were cultured on deoxycholate–citrate agar before and after enrichment in selenite-F medium for 24 hr. at 37° C. The plates were incubated for 24 hr. at 37° C. and then examined for the presence of salmonellae. Portions of organs weighing about 2 g. were first ground with sterile sand in a mortar. Brilliant green broth (Smith, 1952) was substituted for selenite-F medium in the search for *S. cholerae-suis* because sodium selenite is very toxic for this serotype; brilliant green agar (Smith, 1952) was also used in addition to deoxycholate–citrate agar for the direct culture of specimens from pigs.

Agglutination tests

The O and H antibody content of sera was estimated by conventional methods (Cruickshank, 1960).

RESULTS

(A) The immunization of mice against *Salmonella dublin* infection

Antisera

The effect of antisera in protecting mice against *S. dublin* infection is illustrated in Table 1. The control mice were given the pooled sera of rabbits before they were used to prepare the antisera that were given to the other mice. The table summarizes the results obtained in three separate experiments each with thirty mice per group and, in all three, antisera prepared against live *S. dublin* had a definite protective effect whereas that prepared against dead *S. dublin* had little or no effect. The serum given to the control mice contained no demonstrable antibodies to *S. dublin*. The anti-O titre of the serum prepared against live *S. dublin* was 1/1600 and the anti-H titre 1/32,000. The corresponding figures for the serum prepared against dead *S. dublin* were 1/3200 and 1/64,000.

In another experiment employing groups of thirty mice, heating to 58° C. for 1 hr. did not have a deleterious effect on the immunity conferred by antiserum prepared against live *S. dublin*. The difference in the mortality pattern between groups given heated and unheated antiserum and the group given normal serum was similar to that shown in Table 1.

Table 2 shows that the degree of immunity conferred by antisera against live *S. dublin* prepared in calves and in rabbits was similar. The H antibody titre of the calf serum, 1/16,000, was similar to that of the rabbit antiserum used in the previous experiments but the O titre, 1/40, was much lower.

Table 1. *The effect of rabbit antiserum in conferring immunity against* Salmonella dublin *infection in mice*

Time after infection (days)	Cumulative percentage mortality in 90 mice given: Antiserum against live *S. dublin*	Antiserum against dead *S. dublin*	Normal serum*
5	1	1	2
6	3	8	19
7	8	16	29
8	13	25	29
9	16	34	38
10	20	40	46
11	22	48	49
12	25	57	58
13	30	64	64
14	32	70	69
15	34	76	71
16	44	83	79
17	48	84	81
18	50	88	83
19	50	91	84

* The normal serum was pooled serum taken from rabbits before they were used for preparing antiserum against the live and dead (heat-killed) *S. dublin*. Sera in 1·0 ml. amounts were given subcutaneously to the mice 2 hr. before oral infection with *S. dublin*.

Table 2. *The effect of calf antiserum in conferring immunity against* Salmonella dublin *infection in mice*

Time after infection (days)	Cumulative percentage mortality in twenty-five mice given: Antiserum against live *S. dublin*	Normal calf serum
6	4	4
7	8	28
8	12	32
9	12	44
10	12	44
11	16	48
12	16	52
13	16	56
14	24	60
15	32	64
16	36	68
17	40	76
18	40	80

The dose of serum employed was 1·5 ml. For other details see Table 1.

Dead vaccines

The immunizing ability of the following dead vaccines prepared from *S. dublin* 188, the fully virulent strain used for challenge, was tested in groups of twenty mice:

(1) Saline suspension of an 18 hr. nutrient agar culture killed by ethanol.

(2) Alum-precipitated bacterial suspensions prepared by the method of Henning (1953).

(3) Saline suspensions of 6 and 18 hr. nutrient agar cultures killed by ultraviolet light, with and without final precipitation with alum.

(4) Saline suspensions of 6 and 18 hr. nutrient agar cultures disrupted by ultrasonic rays and then rendered sterile by passing through a membrane filter.

(5) Nutrient broth cultures, 18 hr. old, killed by (*a*) heating to 56° C. for ½ hr., (*b*) the addition of 0·06 % formaldehyde.

(6) Nutrient broth cultures, 6 hr. old, killed by: (*a*) neomycin, 500 μg./ml.; (*b*) polymixin, 500 μg./ml.; (*c*) penicillin, 500 units/ml.; (*d*) a saturated aqueous solution of furazolidone.

(7) A 6 hr. nutrient broth culture lysed by bacteriophage and rendered bacteria-free by filtration.

On challenge, the mortality rate in the control mice in these experiments was 90 % and that in the vaccinated groups varied from 70 to 100 %. In a final experiment in which the dose of the alum-precipitated vaccine was increased from the customary 0·1 to 0·25 ml., the dose employed by Henning (1953), the mortality rate in the vaccinated group of twenty-five mice was 52 % and in the similar-sized control group was 80 %.

Live vaccines

Live vaccines were prepared by a variety of methods. These included prolonged incubation of the initially fully virulent strains of *S. dublin* 188, in broth and liquid synthetic media held at different temperatures with and without occasional subculture. *S. dublin* O antiserum and bacteriophages were added to some of the cultures. At intervals, subcultures were made on plates of nutrient agar and colonies selected either at random, according to their morphology or according to their reaction to the slide acriflavine test. Those retained for assessment as vaccines were subcultured five times, single colonies being selected on each occasion, the final culture then being maintained on Dorset egg medium at 5° C. and by freeze-drying.

In all, fifty-five vaccines, some rough and some smooth, were tested on groups of ten to twenty mice. Most of them were rejected because of excessive virulence, because they reverted from avirulent to virulent during this initial test or during subsequent passage, or because they produced little or no immunity. Further tests revealed one vaccine, no. 17 A, to be the most promising. However, when this vaccine was later shown to possess little or no immunogenicity for calves, attention was re-directed to some of the vaccines initially rejected because of excessive virulence for mice and one of them, no. 51, was found to be avirulent and immunogenic for calves.

All the studies reported below are concerned with the assessment of the immunity conferred against *S. dublin* infection by the two vaccines 17A and 51 and by *S. gallinarum*, 9, and its two variants, 9S and 9R, and by *S. pullorum*.

Characteristics of vaccines 17A and 51

Vaccine 17A was obtained by submitting *S. dublin* 188 to fourteen passages of 3–10 days duration in nutrient broth at 18° C. and finally selecting a rough colony. Vaccine 51 was obtained by spreading an 18 hr. broth culture of 188 over the surface of a dried nutrient agar plate and placing a drop of a suspension of salmonella anti-O phage no. 1 (Felix & Callow, 1943) upon it. The plate was incubated at 37° C. for 24 hr. and a phage-resistant colony from within the zone of lysis selected.

Colonies of 17A and 51 had a rough appearance and suspensions agglutinated immediately and completely when submitted to the slide acriflavine test. Broth cultures of 17A, after incubation at 37° C. for 18 hr., consisted of a coarse granular deposit and a clear supernatant fluid; the deposit in the case of 51 was finer and more powdery and the supernatant fluid was turbid. On slide testing, 17A slowly agglutinated spontaneously in normal saline but 51 formed a stable suspension. The biochemical reactions of 17A and 51 were those of 188 itself, a typical strain of *S. dublin*.

The virulence for mice of live S. dublin, S. gallinarum *and* S. pullorum *vaccines*

The virulence for mice of vaccines 17A and 51, their parent strain of *S. dublin* 188, vaccines 9S, 9R and their parent strain of *S. gallinarum* 9, and *S. pullorum* is shown in Table 3, from which it can be seen that 17A possessed very little virulence and that vaccine 51 and, to a lesser extent, 9S had a considerable degree of virulence which was, however, less than that of their parent strains. The lesions found in mice that died after vaccination with 17A, 51 and 9S were the same as those produced by fully virulent salmonellae, and included the characteristic white necrotic regions in the liver. The cultures isolated from the dead mice appeared to have undergone no change and further passage experiments did not show any increase in virulence.

The immunizing effect in mice of live vaccines against S. dublin

The immunity to *S. dublin* infection of mice that survived vaccination with different cultures is illustrated in Table 4. Those vaccinated with 51 were completely immune and those vaccinated with either 17A or *S. gallinarum* 9S had a substantial immunity; the mice vaccinated with either *S. gallinarum* 9R or *S. pullorum* were fully susceptible to infection with *S. dublin*.

The effect of vaccinating mice with 17A at different times before and after challenge with S. dublin

The immunity possessed by groups of forty mice vaccinated with 17A at different times before and after challenge with *S. dublin* is illustrated in Table 5. An appreciable immunity was detected in mice vaccinated before and on the day

Table 3. *The virulence for mice of live vaccines*

Time after injection (days)	Cumulative percentage mortality of mice given: *S. dublin* 17A (255)*	*S. dublin* 51 (109)	*S. dublin* 188 (20)	*S. gallinarum* 9S (125)	*S. gallinarum* 9R (36)	*S. gallinarum* 9 (20)	*S. pullorum* (30)
2	0	0	5	1	0	0	0
3	1	1	10	1	0	0	0
4	1	6	55	1	0	0	0
5	2	21	85	2	0	0	0
6	3	39	100	14	0	15	7
7	3	44	100	22	0	40	7
8	5	55	100	27	0	50	7
9	6	62	100	29	0	50	7
10	6	70	100	29	0	50	10
11	6	72	100	29	0	50	10
12	6	72	100	29	0	50	10
13	6	72	100	29	0	50	10
14	6	77	100	29	0	50	10
15	6	79	100	29	0	50	10
16	6	81	100	29	0	50	10
17	6	81	100	29	0	50	10
18	6	82	100	29	0	50	10

The dose of each vaccine given by subcutaneous injection was approx. 5×10^7 viable bacteria.

* The numbers of mice per group are shown in parentheses.

Table 4. *The immunizing effect of live vaccines against* Salmonella dublin

Time after challenge with *S. dublin* 188 (days)	Cumulative percentage mortality in mice vaccinated with: *S. dublin* 17A (144)*	*S. dublin* 51 (20)	*S. gallinarum* 9S (41)	*S. gallinarum* 9R (26)	*S. pullorum* (27)	None (126)
6	1	0	0	4	4	6
7	3	0	3	16	4	15
8	7	0	8	16	16	24
9	8	0	8	20	26	35
10	14	0	15	28	30	41
11	17	0	18	44	48	48
12	19	0	23	44	48	55
13	19	0	23	44	55	61
14	20	0	25	48	55	66
15	21	0	25	54	60	68
16	21	0	25	63	63	70
17	21	0	25	69	67	73
18	23	0	25	71	70	75

* The numbers of mice per group are shown in parentheses.

of challenge, although it was never as good as in mice vaccinated with 17A at 3 weeks before challenge (Table 4). The groups vaccinated 3 and 5 days after challenge appeared to be unaffected by vaccination, their mortality pattern closely resembling that of the control group.

Table 5. *The effect of vaccinating groups of* 40 *mice with* Salmonella dublin *live vaccine* 17*A at different times before and after oral challenge with* Salmonella dublin

	Cumulative percentage mortality in mice vaccinated on the following days in relation to challenge									
Time after challenge (days)	Before						After			
	10	7	5	3	1	0	1	3	5	Controls
6	3	8	0	0	3	3	5	3	8	8
7	5	8	3	5	10	12	23	28	30	25
8	10	13	5	8	25	15	38	38	35	38
9	13	20	8	10	25	18	43	43	38	43
10	13	30	13	23	28	25	45	48	38	60
11	15	38	13	23	30	25	58	63	45	68
12	18	43	15	30	33	30	58	63	53	78
13	20	43	15	35	35	30	60	70	63	85
14	25	45	18	40	35	33	60	70	65	87
15	28	45	25	43	38	38	63	75	70	87
16	33	45	25	45	38	43	65	80	78	87
17	33	45	33	50	43	45	68	83	80	87
18	38	45	40	53	50	50	68	83	83	90

Table 6. *The effect of different doses of* Salmonella dublin *live vaccine* 51 *in conferring immunity against* S. dublin *infection in mice*

	Vaccinal dose (viable organisms)			
	50,000	5000	500	0
No. of mice	30	27	25	23
Percentage died from vaccination	57	48	8	0
No. of survivors challenged	13	14	23	23
Percentage died from challenge	0	0	13	83

The immunizing ability of vaccine 17*A against heterologous strains of* S. dublin

When twenty mice vaccinated with 17A were challenged with a strain of *S. dublin* epidemiologically unrelated to 188, five died compared with seventeen of twenty control mice. When the experiment was repeated using another challenge strain, five vaccinated and sixteen control mice died.

The influence of dose size on the virulence and immunizing ability of vaccine 51

Since vaccine 51 in its usual dose of 5×10^7 viable organisms killed a considerable proportion of the mice injected with it, the virulence and immunizing ability of smaller doses were assessed. The results (Table 6) indicated that doses much smaller than the customary vaccinal dose were lethal for mice and that only when the numbers of viable organisms were reduced to 500 was the virulence greatly

decreased, a procedure that was accompanied by a slight mortality on challenge with the fully virulent strain.

The presence of agglutinins in the sera of mice vaccinated with 17*A and* 51

No O-agglutinins to *S. dublin* were detected by tube tests in a 1 in 10 dilution of the sera of fifteen mice 3 weeks after vaccination with 17A or 51. The H titres of the sera varied from 1/160 to 1/640.

(B) The immunization of mice against *Salmonella cholerae-suis* infection

Dead vaccines

Only one dead vaccine was tested. This was an alum-precipitated vaccine prepared from *S. cholerae-suis* 195 and used in the manner described by Henning (1953) for *S. dublin*. Of twenty vaccinated mice, fourteen died after challenge; seventeen of twenty control mice died.

Live vaccines

These were prepared in a similar manner to the live *S. dublin* vaccines except that the parent strain was *S. cholerae-suis* 195. Eight vaccines were tested in mice and two, nos. 3 and 6, were retained for further study because they satisfied the criteria initially sought for in the *S. dublin* vaccines except that they were still appreciably virulent for mice.

Characteristics of vaccines 3 *and* 6

Vaccine 3 was produced by the same procedure as the *S. dublin* vaccine 17A and vaccine 6 by the same procedure as vaccine 51. Both vaccines 3 and 6 were judged to be rough on colonial appearance. Broth cultures of vaccine 3, after 24 hr. at 37° C., consisted of a powdery deposit and a clear supernatant fluid; a powdery deposit was also present in the case of vaccine 6 but the supernatant fluid was turbid. Suspensions of both vaccines agglutinated immediately and completely when submitted to the slide acriflavine test. They also agglutinated slowly in normal saline, vaccine 6 being the slower. The colonies of 3 and 6 on deoxycholate–citrate agar were smaller than those of their parent strain 195. After 24 hr. incubation on this medium at 37° C., colonies of 195 had a diameter of 2 mm., whereas the diameter of those of 3 and 6 were 1 and 0·5 mm. respectively. After 48 hr. incubation the colonies of 195 were flat with an uneven edge and 3–4 mm. diameter; those of 3 and 6 had a 'poached egg' appearance and their diameters were approximately 1·5 and 1 mm. respectively.

The virulence and immunizing ability of vaccines 3 *and* 6 *in mice*

Experiments to assess the virulence and immunizing ability of vaccines 3 and 6 are summarized in Table 7, from which it can be seen that both vaccines possessed a considerable degree of virulence for mice, greater in 3 than in 6, and that the survivors had a considerable degree of immunity against oral infection with *S. cholerae-suis* 195. The mortality pattern from vaccination and challenge was

similar to that shown previously in the case of the *S. dublin* vaccine 51 and *S. dublin* 188 respectively. All strains isolated from mice dead from vaccination were rough.

Table 7. *The virulence and immunizing ability of* Salmonella cholerae-suis *vaccines* 3 *and* 6 *in mice*

	Vaccinated with			
	3	6	195*	Unvaccinated
No. of mice	70	139	30	80
Percentage died from vaccination	63	44	100	0
No. of survivors challenged	26	78	0	80
Percentage died from challenge	15	14	0	84

* 195 is the fully virulent strain from which vaccines 3 and 6 were derived. It was also the strain used for oral challenge.

Table 8. *The effect of vaccinating groups of* 20 *mice with* Salmonella cholerae-suis *vaccine* 6 *at different times before and after oral challenge with* S. cholerae-suis

	No. of days vaccinated in relation to challenge									
	Before						After			
	10	7	5	3	1	0	1	3	5	Unvaccinated
Percentage died from vaccination*	40	35	55	35	5	0	0	0	0	0
Percentage died from challenge*	5	15	20	25	80	80	100	100	95	75
Total % died	45	50	70	60	85	80	100	100	95	75

* Assessed by performing the acriflavine test on the colonies isolated from the livers of the dead mice.

Table 9. *The virulence and immunizing ability of* Salmonella cholerae-suis *vaccines when administered orally to mice*

	Vaccinated with		
	3	6	Unvaccinated
No. of mice	20	20	20
Percentage died from vaccination	50	30	0
No. of survivors challenged with *S. cholerae-suis* 195	10	14	20
Percentage died from challenge	15	15	85

The vaccines were administered orally in exactly the same manner as the challenge dose was 3 weeks later.

The effect of vaccinating mice with vaccine 6 *at different times before and after challenge with* S. cholerae-suis

The numbers of mice that died in groups of twenty given vaccine 6 at different times before and after challenge with *S. cholerae-suis* 195 is shown in Table 8. The reaction to the acriflavine test of the strain isolated from the livers of the dead mice was the factor used to decide whether death was due to vaccination or challenge. On this basis, most of the dead mice that had been vaccinated 3 or more days before challenge were considered to have died from vaccination and all

but one of the dead mice vaccinated after this time had died from the challenge infection. As expected, the total mortality was least in the mice vaccinated 10 days before challenge. In those vaccinated 1–5 days after challenge it was higher than in the unvaccinated controls.

The effect of oral administration of vaccines 3 *and* 6

The results of giving vaccines 3 and 6 orally to mice 3 weeks before challenge with *S. cholerae-suis* is shown in Table 9. The vaccines were administered in exactly the same manner as the challenge strain. Both vaccines had a considerable lethal effect and the survivors exhibited a reasonably high level of immunity.

The presence of agglutinins in the sera of mice given vaccines 3 *and* 6

No O-agglutinins to *S. cholerae-suis* were detected by tube tests in a 1 in 10 dilution of the sera of thirteen mice 3 weeks after vaccination with 3 or 6.

Table 10. *The immunity conferred by live vaccines against challenge in mice with heterologous species of* Salmonella *and other bacteria*

Vaccine	Challenge organism	No. of vaccinated mice challenged	Mortality following challenge (%)	No. of unvaccinated mice challenged	Mortality following challenge (%)
S. cholerae-suis 6	*S. dublin* 188	29	3	24	96
S. dublin 51	*S. cholerae-suis* 195	37	3	20	55
S. dublin 51	*S. typhi-murium*	27	4	21	86
S. dublin 51	*Erys. rhusiopathiae*	25	100	25	100
S. dublin 51	*E. coli*	22	69	19	88

The immunity conferred by the live vaccines against infections in mice with heterologous salmonella serotypes and other bacteria

The results of administering live vaccines to mice and 3 weeks later challenging the immunity of the survivors against bacteria other than the one from which the vaccine they had been given was derived is illustrated in Table 10. The dose of vaccine was reduced from the usual 5×10^7 living organisms to 5×10^6 and this resulted in a vaccinal mortality of from 33–45 %. The immunity of the survivors to salmonella infection was determined in the usual way. That against *Escherichia coli* was determined by the intraperitoneal injection of 10^8 viable organisms of a 24 hr. nutrient broth culture, approximately 3 times the LD 50, and that against *Erysipelothrix rhusiopathiae* by the subcutaneous injection of 10^7 viable organisms of a 24 hr. broth culture. The *S. cholerae-suis* vaccine 6 conferred a high degree of immunity against *S. dublin* and so did *S. dublin* vaccine 51 against *S. cholerae-suis* and *S. typhi-murium*. Vaccine 51 produced no apparent immunity against *Erys. rhusiopathiae*, the mice in the vaccinated and the control groups exhibiting a similar mortality pattern, deaths commencing on the third day and concluding on the fifth day after infection. It also produced no apparent immunity against infection with *E. coli*.

(C) THE IMMUNIZATION OF CALVES AGAINST *SALMONELLA DUBLIN* INFECTION

Experimental studies

The results of experiments on the efficacy of antiserum and vaccines 17A, 51 and 9S in protecting calves against *S. dublin* infections are summarized in Table 11. The O and H titres of the antiserum, which was prepared in calves given live cultures of *S. dublin* 188, were 1/40 and 1/16,000 respectively. None of the calves showed any signs of ill-health after vaccination. Appetite and body temperature were unaltered and the faeces remained normal in consistency and were not found

Table 11. *The immunizing ability of live vaccines and antisera against* Salmonella dublin *infection in calves*

Immunizing agent	No. of calves used	Cumulative mortality on the following days after challenge with *S. dublin* 188						No. of survivors
		2	3	4	5	6	7	
Antiserum* (450 ml.)	5	1	2	3	3	4	5	0
Vaccine 17 A	8	0	2	6	6	6	8	0
Vaccine 9 S	7	0	0	0	2	4	4	3
Vaccine 51	5	0	0	0	0	1	1	4
Vaccine 51 + 9 S	2	0	0	0	0	0	0	2
Controls	14	4	10	13	13	13	14	0

* Prepared in calves against live *S. dublin* and given 2 hr. before challenge.

to contain the vaccinal or any other salmonellae. At 3 weeks after vaccination no O antibodies against *S. dublin* were detected in the sera of the calves. The sera of those vaccinated with 17A and 51, but not with the non-motile 9S, possessed H antibody titres of 1/400–1/1600. No *S. dublin* O or H antibodies were found in the sera of the unvaccinated calves. Within 1–3 days of challenge all the calves appeared unwell, their body temperature rose to 104–107° F., their appetites were impaired, they had diarrhoea and their faeces contained *S. dublin*. These signs were most prominent in the control calves and in those given antiserum or vaccine 17A. Apart from some possible slight difference in survival time, the disease appeared equally severe in these three groups of calves. In the others the disease was less severe, particularly in those vaccinated with 51 or 51 and 9S. Although only two calves were given both 51 and 9S there was no real evidence to indicate that a combination of both vaccines produced a better immunity than 51 alone. Three weeks after challenge all the surviving calves appeared reasonably well and *S. dublin* was not found in their faeces. Two of the calves vaccinated with 51 and one each vaccinated with 9S or 9S and 51 were killed at this time; salmonellae were not isolated from their organs.

The results of a further experiment to assess the immunizing ability of vaccines 51 and 9S are summarized in Table 12. In this experiment the calves were not Ayrshires, the breed used in the previous experiment, but male Friesians reared intensively for beef production. There were selected for the final experiment,

despite their cost, because clinical *S. dublin* infection is most common in intensive beef production units. These calves had been maintained on a diet consisting solely of solid concentrated food for a week before challenge when approximately 6 weeks of age, having been vaccinated 3 weeks previously. Neither the vaccinal strains nor any other forms of salmonellae were found in the faeces of these animals before challenge and they all remained in a good state of health. Only H antibodies against *S. dublin* were found in the sera of those vaccinated with 51. The course of the disease after challenge was less severe in the control animals than in those used in the previous experiment. Not only was the death-rate lower but the survival time of those that died was much longer. None of the animals died in the early bacteraemic phase as most of the control calves did in the previous experiment. Most that died became emaciated and suffered from a diarrhoea in which the faeces often contained pieces of necrotic mucous membrane. The disease was definitely most mild in those given vaccine 51 and the death rate was nil.

S. dublin was only isolated from the faeces of one of the surviving calves after the 17th day of infection, at which time most of them were in reasonably good condition.

Table 12. *The immunizing ability of live vaccines against* Salmonella dublin *infection in intensively-reared calves*

Vaccine	No. of calves used	Cumulative mortality on the following days after challenge						No. of survivors
		7	8	9	10	11	11+	
51	10	0	0	0	0	0	0	10
9 S	10	1	1	2	2	2	3	7
None	10	0	1	2	3	5	6	4

Field studies

Field studies were performed from October 1963 to August 1964 in an intensive beef production unit that had previously experienced a considerable amount of clinical *S. dublin* infection. The calves, male Friesians, had been born on farms in south-west England and were brought in batches to the unit in Essex when approximately 1 week old. One-third of the calves were vaccinated in the usual manner with 51, one-third with 9S and the remainder with a heat-killed broth culture of a coagulase-negative staphylococcus; the owner of the unit was not made aware of the particular vaccine any calf received. In the first half of the experiment the calves were vaccinated at 3 weeks of age but in the second half, owing to the fact that clinical *S. dublin* infection began to occur in the young age groups, they were vaccinated on the day after their arrival at the unit. None of them showed any signs of ill-health that could be attributed to vaccination. Subsequently the owner recorded the rectal temperature and took a rectal swab of all animals that became unwell. Immediately after this the owner was permitted, on economic grounds, to treat these animals with furazolidone, which he usually did. The swab was brought to the laboratory for bacteriological examination. In the absence of any other diagnosable disease, all the ill animals from which

S. dublin was isolated from the faeces were recorded as suffering from clinical *S. dublin* infection. Clinically, these animals formed a fairly clear-cut group in that they were dull, disinclined to move or eat, had temperatures of 104–107° F. and usually had diarrhoea. The results (Table 13) showed that, apart from calves that developed the clinical disease within 1 week of vaccination, vaccine 51 and, to a lesser extent, 9S had a beneficial effect on reducing the incidence of clinical *S. dublin* infection. The vaccination history of sick calves from whose faeces *S. dublin* was not isolated but whose rectal temperatures were 104·5° F. or higher was also analysed; of 36, seven had been vaccinated with vaccine 51, eight with 9S and 21 with the dead staphylococcus.

Table 13. *The incidence of clinical* Salmonella dublin *infection in vaccinated calves in an intensive beef unit*

Vaccine	No. of calves vaccinated	No. that developed infection in relation to vaccination	
		Within 1 week	After 1 week
51	312	5	3
9 S	312	2	9
Dead coagulase-negative staphylococcus	312	5	35

On six occasions during the period November 1963 to June 1964 the faeces of most of the healthy calves under 6 months of age in the unit were examined for the presence of *S. dublin*. Smooth strains of *S. dublin* were found in sixty-three (4·9 %) of the 1293 rectal swabs examined. There was little variation in the incidence of positive swabs from occasion to occasion. Ten of them were from calves vaccinated with 51, twenty from calves vaccinated with 9S and thirty-three from calves vaccinated with the dead staphylococcus. Rough strains of *S. dublin* were found in the faeces of one calf vaccinated with 51 and in one vaccinated with the dead staphylococcus; they were not found at repeat examinations two days later.

(D) THE IMMUNIZATION OF PIGS AGAINST *SALMONELLA CHOLERAE-SUIS* INFECTION

Experimental studies

The results of challenging the immunity to *S. cholerae-suis* infection of pigs given either one of the live vaccines 3 and 6 or a dead alum-precipitated vaccine are summarized in Table 14. None of the pigs showed any signs of ill-health after vaccination, their appetites were unimpaired and they continued to gain weight in the normal manner. The highest body temperature recorded in the individual pigs in the 3 weeks before challenge varied from 103 to 105° F. (median 104° F.) in the unvaccinated control group and in the vaccine 3 group and from 102·5 to 104·8° F. (104·1° F.), in the vaccine 6 group. During this period neither the vaccinal strain nor any other form of *S. cholerae-suis* was isolated from the faeces of the pigs. Immediately before challenge, the sera of four pigs in each live vaccine

group and the control group were examined for antibodies against the strain of *S. cholerae-suis*, 195, with which they were to be challenged. No O antibodies were found in any of the sera diluted 1 in 10. No H antibodies were found in the sera of the control pigs; the sera of the pigs in the two vaccinated groups contained similar levels of H antibodies, the titres ranging from 1/200 to 1/3000.

Table 14. *The immunizing ability of vaccines against* Salmonella cholerae-suis *infection in pigs*

Vaccine	No. of pigs used	Cumulative mortality on the following days after challenge									No. of survivors		
		5	6	7	8	9	10	11	12	13	With severe lesions	With mild lesions	With no lesions
3	12	0	0	0	1	1	1	1	1	1	0	0	11
6	12	0	0	0	0	0	0	0	0	0	0	1	11
Dead alum-precipitated*	4	0	0	0	1	1	1	1	1	1	2	0	1
None	12	1	2	2	2	3	4	4	4	7	4	0	1

* Prepared according to the method of Henning (1953).

Within 2 days of challenge all the pigs appeared unwell. They refused food, some vomited and their body temperatures varied from 105 to 108° F. The body temperatures of the pigs given live vaccines remained above 105° F. for 1–3 days and their appetites were impaired for up to 6 days. However, with the exception of the one pig given vaccine 3 that died and the one pig given vaccine 6 which had lesions when killed at the end of the experiment (Table 14), the general health of the pigs given live vaccines was greatly improved at 7 days after challenge and by 14 days they appeared normal. By contrast, the unvaccinated control pigs had very high body temperatures for 3–4 days and, with the exception of one that was normal at the end of the experiment, the remaining eleven ate little or no food and their general condition continued to deteriorate until they died or were killed at the termination of the experiment, the survivors by then being in an extremely emaciated condition and unlikely to recover. All these survivors are recorded in Table 14 as having severe lesions which principally consisted of necrotic enteritis involving the whole of the large intestine. The designation 'mild lesions' implied the presence of small areas of necrotic enteritis in the large intestine which were resolving. Although only four pigs were vaccinated with the dead alum-precipitated vaccine it was apparent that their immunity was much less than that in the pigs given vaccines 3 and 6.

At the end of the experiment, *S. cholerae-suis* was not isolated by direct culture from the liver, bile, spleen or from a mesenteric lymph node of eight pigs examined that had been given vaccine 3 or of eight pigs examined given vaccine 6. Preliminary enrichment of approximately 2 g. of each of these materials revealed the presence of *S. cholerae-suis* in the mesenteric lymph node of one of the pigs given vaccine 6.

Field studies

From November 1963 to June 1964 field studies were conducted on a very large pig farm some 300 miles from the laboratory, a farm in which cases of clinical *S. cholerae-suis* infection of a bacteraemic type had been occurring for several years. On this farm, all weaning was performed on one day only of every week, the weaned pigs, approximately 8 weeks of age, then being mixed together. One-third of all the pigs weaned in any week were given vaccine 3, another third vaccine 6 and the remainder were left as unvaccinated controls. No complaints were received of any ill effect of vaccination. No treatment was given to any pigs that became unwell and the spleens of all that died were sent to our laboratory by post. Pigs from whose spleens *S. cholerae-suis* was isolated in plentiful culture were recorded as having died from *S. cholerae-suis* infection. The results are summarized in Table 15, from which it can be seen that both vaccine 3 and vaccine 6 had a controlling effect on the incidence of fatal cases of *S. cholerae-suis* infection.

Table 15. *The effect of vaccination on the incidence of fatal* Salmonella cholerae-suis *infection in a herd of pigs*

		No. that died from	
Vaccine	No. of pigs vaccinated	All causes	*S. cholerae-suis* infection
3	384	32	9
6	384	28	10
None	384	70	40

The deaths attributed to *S. cholerae-suis* infection occurred from 9 to 68 (median 22) days after vaccination. Several pigs, including unvaccinated ones, died, presumably from other causes, within 1 week of the vaccination time; this mortality was no higher than at other periods. A rough strain of *S. cholerae-suis*, probably vaccine 3, was found in plentiful culture in the spleen of one pig that died 5 days after it had been given this vaccine. Neither of the two vaccines was isolated from any of the other spleens by direct culture.

DISCUSSION

A great deal of doubt exists as to the relative importance of cellular and humoral immunity in salmonella infection. Although the protection produced in mice in the present work by a large dose of antiserum prepared in rabbits or calves against live *S. dublin* was far from complete and inferior to that produced by live vaccines it was sufficient to indicate that humoral immunity may play some part in salmonella infection. The administration of antiserum, however, had very little practical application in *S. dublin* infection in calves. The fact that both antiserum prepared against dead *S. dublin* and dead vaccines had very little protective effect on mice suggests that the immunogenic factors present in the live bacteria were destroyed by the killing processes. The negative results with these materials which either contained or gave rise to considerable amounts of O and H antibodies and the fact that the live rough *S. dublin* and *S. cholerae-suis* vaccines provoked no O

antibodies and the live smooth *S. gallinarum* vaccine 9S provoked no H antibodies yet all produced a reasonably good immunity provides confirmatory evidence for the view that the conventional O and H antigens are not concerned in salmonella immunity. The observation that mice surviving vaccination with the *S. choleraesuis* variant 6 were resistant to *S. dublin* infection and mice surviving vaccination with the *S. dublin* variant 51 were resistant to *S. cholerae-suis* infection (Table 10) indicates that *Salmonella* species bearing little resemblance as far as ordinary *in vitro* antigen–antibody tests are concerned may be closely related immunogenically. It cannot be argued that the immunity produced by these variants was non-specific in character because the mice vaccinated with variant 51 were fully susceptible to both *Escherichia coli* and *Erysipelothrix rhusiopathiae* infections. On the other hand, immunogenic factors were not common to all members of the *Salmonella* groups since the mice vaccinated with *S. pullorum* were fully susceptible to *S. dublin* infection yet *S. pullorum* and other members of the O '9' group immunized chickens against *S. gallinarum* infection (Smith, 1956*b*).

Mice that survived vaccination with variant 51 were more resistant to challenge with the fully virulent *S. dublin* 188 than were those vaccinated with variant 17A (Table 4). This might be because the severer infection that followed vaccination with 51 constituted a greater immunogenic stimulus than the milder infection that followed vaccination with the less virulent 17A. In view of the greater tolerance of calves than mice to these vaccines, it is conceivable that the failure of 17A to protect calves against challenge with 188 (Table 11) was because the infection produced in them by 17A was too mild to constitute an adequate immunogenic stimulus whereas the infection produced in them by 51, although nothing like as severe as that produced in mice, was sufficient to produce a reasonable immunity. It is noteworthy in this respect that the reduction of the dose of variant 51 in mice to a point at which the vaccinal mortality was greatly reduced (500 organisms) was accompanied by an immunity to challenge with 188 which, although of a high order, was less than that following the administration of larger doses (Table 6).

The experimental and field studies in calves and pigs indicated that the live vaccines may have a practical application in the control of salmonella infection in these species. The results point to 51 being better than 9S in preventing *S. dublin* infection in calves and, since it was highly effective against experimental infection in mice with *S. typhi-murium*, it is conceivable that 51 may have a beneficial effect in controlling disease in calves caused by this organism—the second most important salmonella type causing disease in these animals. The studies revealed little difference between vaccines 3 and 6 in controlling experimental and natural *S. cholerae-suis* infection in pigs. However, since vaccine 3 was found in plentiful culture in the spleen of one dead pig in the field trial—the significance of which is in doubt, particularly as the spleens had been sent to the laboratory by post—vaccine 6 is to be preferred in further studies.

The rapidity with which immunity developed in mice after injection of the live vaccine 17A resembled the 'interference' type phenomenon reported after vaccination with 9S in experimental *S. gallinarum* infection in chickens (Smith, 1956*a*). Vaccine 17A never produced any ill effect related to its time of administration.

An ill effect was noted when mice were injected with vaccine 6 shortly after oral infection with *S. cholerae-suis* and this was probably associated with the considerable degree of virulence of vaccine 6 for these animals. Since vaccine 51 was avirulent for calves and vaccines 3 and 6 avirulent for pigs, it is unlikely that these vaccines would cause harm when administered to infected animals during outbreaks of salmonella infection; they might well be beneficial. Vaccine 51 was, in fact, administered to some infected calves during the field studies; the losses were certainly no higher than in the controls. However, this point and others can only be settled by further use of these vaccines.

SUMMARY

1. Antisera prepared in rabbits or calves against live *Salmonella dublin* gave mice some degree of protection against oral infection with this organism. Both antiserum prepared against heat-killed *S. dublin* and dead vaccines prepared in a variety of ways produced little or no immunity.

2. A rough variant of *S. dublin* of low virulence for mice, no. 17A, produced a reasonably good immunity against oral infection with *S. dublin* in mice but not in calves. Mice that survived injection with another rough variant that possessed a considerable degree of virulence for these animals, no. 51, were immune to oral infection with *S. dublin*. Experimentally and naturally, this variant and, to a lesser extent, 9S, a smooth variant of *S. gallinarum* of reduced virulence, produced an appreciable degree of immunity in calves against *S. dublin* infection; none of the calves injected with these variants showed any signs of ill-health as a result.

3. Two rough variants of *S. cholerae-suis*, nos. 3 and 6, possessed a considerable degree of virulence for mice; those that survived were resistant to oral infection with *S. cholerae-suis*. Experimentally and naturally, both variants produced an appreciable degree of immunity in pigs.

4. Mice that survived vaccination with the rough *S. cholerae-suis* variant no. 6 were resistant to oral infection with *S. dublin*. Those that survived vaccination with the rough *S. dublin* variant no. 51 were resistant to oral infection with *S. cholerae-suis* and *S. typhi-murium*; they were fully susceptible to parenteral administration of *Escherichia coli* and *Erysipelothrix rhusiopathiae*.

5. Vaccination with *S. cholerae-suis* variants 3 and 6 and *S. dublin* variant 51 provoked the formation of H but not O antibodies. These variants were never found to mutate from rough to smooth *in vitro* or *in vivo*.

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ADDENDUM

From 1 July 1964 all 575 calves brought into the beef production unit were vaccinated with vaccine 51. By 30 December only six had shown signs of clinical *S. dublin* infection that could be associated with vaccine failure; three of them had also been suffering from 'virus' pneumonia. The last case occurred on 18 November. On 2 September 120 of the younger calves in the unit were examined and smooth strains of *S. dublin* were isolated from the faeces of ten and rough strains from the faeces of two; all twelve calves appeared healthy. On 22 December all 422 calves in the unit were examined and a smooth strain of *S. dublin* was isolated from the faeces of one calf, a persistent excretor after clinical infection, and rough strains from two. The rough strains isolated from calves in this unit were probably strain 51 itself; they were never found in calves at repeat examinations.

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Nutrition in the Caribbean

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During the winter of 1963–64 I made a visit of about three months to the Caribbean as a 'Special Consultant on Nutrition' to the Pan American Health Organization, which is the Regional Office of the World Health Organization for the Americas (PAHO/WHO), and to the Food and Agriculture Organization of the United Nations (FAO). In effect I was asked to study the nutritional problems of the area and assess their relative importance, to observe what is now being done to attack these problems, and to recommend future action. The following territories were visited, in the order indicated: Jamaica, Puerto Rico, St Thomas, Surinam, British Guiana, Grenada, Barbados, St Lucia, Martinique, Dominica, Antigua, St Kitts, Trinidad and Curaçao. Second visits were made to Puerto Rico and Jamaica.

A lengthy report was submitted to the sponsoring organizations. This paper consists mainly of parts of the report dealing with the public health aspects of nutrition in the area.

SOME BACKGROUND

The state of nutrition of any population is determined by many agricultural, economic and social factors. A short account will be given of certain aspects of life in the Caribbean with a bearing on problems of nutrition. In this area the background is unusual and in some respects unique.

The demographical situation is the familiar one found today in most tropical areas, i.e. there is a persisting high birth-rate and a falling death-rate, resulting in rapid population growth. Children under 15 represent 41 % of the total population, as compared with 23 % in the United Kingdom. In the islands the density of population is high, averaging about 500 per square mile, with a peak figure of about 1400 in Barbados. Population pressure is reflected in extensive emigration, when and where opportunities for emigration are available.

Land under cultivation is devoted mainly to cash crops for export, notably sugar and bananas, so that all the islands are heavily dependent on imported food. Some, like Curaçao, and St Thomas in the U.S. Virgin Islands, produce almost no food for local consumption. Others, such as Jamaica, Puerto Rico and Trinidad, produce some of their own food, but only a fraction of requirements. Even the mainland country of Surinam imports much of its food. While in many territories there are possibilities of developing the production of valuable foods such as legumes, vegetables and fish for local use, dependence on imports for a large proportion of calorie supplies is an irremovable feature of the area as a whole.

Governments are naturally eager to promote the local cash crops, since the economy of most territories depends on them, and the efforts of agricultural and other departments are channelled in this direction. Marketing facilities which would encourage smallholders to increase the production of foods such as vegetables and milk are, in general, lacking. Moreover, locally produced foods everywhere face increasing competition from food imports, which are of good quality, and relatively cheap through modern developments in food processing, storage and transport. The result of all this is that the production of foods for local consumption is steadily declining rather than increasing in many territories.

The economic situation in the Caribbean has improved within recent years, as is shown by the rising per caput annual income. Tourists and bauxite are among the important sources of greater prosperity. There are, however, territories which have as yet shared little in the general advance. Equally, within the territories themselves, including the more prosperous of these, sections of the population remain extremely poor whatever the average rise in per caput income. There is also a growing population housed in shanties around towns, liable to unemployment and living in wretched conditions even when times are good. Children admitted to hospital for malnutrition often come from these areas.

Among social features, family structure is of great significance. The fecundity rate, i.e. the average number of children born per 100 women in the reproductive period, is remarkably high. Throughout the area, illegitimate births outnumber legitimate births: to give examples, in Grenada, in 1961, 2499 births out of 3691 were illegitimate, while in Barbados (1960) the corresponding numbers were 4187 and 6754; in St Lucia, about 97 % of first babies of women under 20 are born out of wedlock. Most women, in both urban and rural areas, are employed outside the home, and return to work soon after bearing a child. Financial support for the family is precarious because fathers tend to leave the vicinity of the home for employment elsewhere. Illegitimacy and the prevailing cultural pattern weaken the role of the father in child-rearing and family life, though they usually do not affect the mother's own sense of responsibility and love for her children. The number of absentee mothers, who leave their children to be looked after by others, is, however, increasing. Grandmothers play an important and sometimes disastrous role in child feeding.

The pattern of family life is thus often inimical to the careful rearing of children. The 1960 Report of the Health Department of St Kitts–Nevis–Anguilla says: 'Lack of love, care and understanding on the part of parents and guardians is destroying many of the infants of the colony'. This, in my view, is altogether too harsh a statement for general application, but its appearance in a health department report is worth noting. A good account of West Indian family and domestic patterns (which have, of course, an historical basis in slavery) was recently given by Sheila Patterson at a Royal Society of Medicine Symposium on 'The health of the coloured child in Great Britain' (1964).

There is a shortage of trained personnel to run technical services (health, education, agricultural, etc.) which is particularly serious in the lower ranks but also affects the senior grades.

MALNUTRITION

Malnutrition in infants and young children is the most serious problem of nutrition in the area. In outlining this problem, the following quotation from a recent PAHO/WHO report (1963) dealing with the Leeward and Windward Islands (St Kitts, Antigua, Dominica, St Lucia, St Vincent, Grenada) makes a good starting point: 'In some of these islands, more than 50 % of the total deaths occur by two years of age. It has been demonstrated that the greatest risk in childhood begins at about 6 months of age and ends at the end of the second year of life. When compared with a North American child the risk of dying for a West Indian child is 25 to 30 times greater during this period of life. . . . The two main causes of death in this age group are gastro-enteritis and malnutrition.'

The figures shown in Table 1 for Grenada and St Kitts, supplied by PAHO, extend this statement.

Table 1. *Deaths in infancy and early childhood in Grenada, St Kitts and the U.S.A.*

	Age-specific death-rates				
Age	Grenada, 1961–62	St Kitts, 1960–62	U.S.A. 1959	Ratio: Grenada/ U.S.A.	Ratio: St Kitts/ U.S.A.
Under 1 month	15·4	29·7	19·0	0·8	1·5
1–2 months	4·7	4·9	3·0	1·5	1·6
3–5 months	10·3	19·4	2·4	4·4	8·1
6–8 months	15·4	18·8	1·2	10·4	15·9
9–11 months	19·2	18·3	0·7	26·2	25·1
12–23 months	33·0	35·9	1·7	18·8	23·3
24–35 months	7·0	7·9	1·0	6·6	7·4
36–47 months	3·0	2·2	0·8	3·9	2·8
48–59 months	1·0	2·4	0·7	1·5	3·5

Mortality statistics from other Leeward and Windward Islands show similar trends; the outstanding fact which they reveal is the peak in mortality between 6 months and 2 years. Its association with malnutrition is supported by morbidity data from St Lucia, where malnutrition was made a notifiable condition in 1962. Between December 1962 and October 1963, 189 cases were notified. The age distribution of these ran parallel with the mortality figures, i.e. the majority fell in the age group 6 months to 2 years. There were, however, twenty-four notifications for infants aged 4–6 months (Lees, 1964).

Deaths ascribed to gastro-enteritis, a major cause of mortality in the Caribbean, as in Central and South America, show a similar trend. The majority of deaths (85 % or over) occur in children under 5, and within this age group there is a concentration of mortality between 6 months and 2 years. While deaths from gastro-enteritis have been considerably reduced in some territories, they remain exceptionally numerous in the Leeward and Windward Islands. In Grenada and Antigua in 1960 and 1962 respectively, gastro-enteritis was the leading cause of death, and much the same situation exists in other islands in this group.

When I was being shown round a ward in the General Hospital in one of the

islands, crowded with miserable children, I noted that the doctor called the ward 'the malnutrition ward' while the matron called it 'the gastro-enteritis ward'. It struck me that since gastro-enteritis and malnutrition in infants and young children are almost inseparable conditions, both clinically and statistically, it would be convenient to think of them as a single syndrome. This would be in line with a statement of Back (1960) based on experience in University College Hospital, Jamaica, that 'most of the babies with malnutrition had some degree of gastro-enteritis on admission and few of the babies with gastro-enteritis were well-nourished' but would carry the association a stage further. Later I discovered that the 'single syndrome' concept has found expression in the term 'weanling diarrhoea', coined by Gordon, Chitkara & Wyon (1963). The term itself is not appealing—though it is difficult to think of a better one—but the concept itself is likely to prove of significance in paediatrics. In the Caribbean it helps in fitting together various facts into a rational pattern.

In the human child, as in the young of some other species, the necessary transition from mother's milk to a mixed 'adult' diet can be a difficult and dangerous process, the gastro-intestinal tract being naturally the main danger point. Breast milk is easily digested and uncontaminated with infectious organisms, so that breast feeding 'protects' against gastro-enteritis; this is true in the Caribbean as elsewhere. The replacement of breast milk by other foods can have various effects. First, it usually means, in poor tropical and subtropical countries, a deterioration in the diet, particularly with respect to protein, because cows' milk and other supplementary foods rich in protein are largely unavailable, or not given. Secondly, the foods introduced may be mechanically unsuited to the infant's immature intestines and hence cause intestinal irritation and diarrhoea. Thirdly, these foods are likely to convey organisms which produce diarrhoea through inflammation of the intestinal walls. Diarrhoea leads in turn to deterioration in nutritional status through impaired utilization of foods which, even if fully utilized, would not meet nutritional needs. Chronic diarrhoea and insufficient nourishment induce marasmus caused by a combination of both. After a certain point is reached, the diarrhoea may reflect the inability of the intestines to absorb food because of atrophy resulting from malnutrition, and, in severe cases of protein-calorie deficiency, atrophy of glands secreting digestive enzymes is likely to impair digestion and absorption still further.

Gastro-enteritis occurs in all degrees, from mild gastro-intestinal disturbance to continuous and profuse diarrhoea with vomiting, leading to dangerous dehydration which calls urgently for fluid replacement if the child's life is to be saved. Research on pathogens has been done in a few places in the Caribbean, notably in Jamaica, where pathogens were isolated from the stools of 18·5% of children with diarrhoea, those most commonly found being *Escherichia coli* and *Samonella* (Back & Brooks, 1962). The literature on this subject is reviewed by Gordon *et al.* (1963) who conclude that 'the infectious agents broadly encountered are much the same, suggesting that clinical variations relate mainly to host resistance and infecting dose'. With regard to 'host resistance', these authors go on to say: 'in the well-nourished child, acute diarrhoeal disease is a brief illness of a few days.

An occasional death occurs, but the issue is decided without delay. The usual result is prompt recovery with little or no after-effects.... Among malnourished children of retarded development, the disease is not ordinarily an isolated episode of acute evolution. Clinical severity often seems less than in the well-nourished child, but the attack tends to persist... episodes recur at relatively short intervals'.

Some further vital statistics and other records from the Caribbean will now be considered. In my view these can best be interpreted in the light of the association between malnutrition and gastro-enteritis.

INFANT MORTALITY

In some territories there has been a striking fall in infant mortality during recent decades. This is shown by the figures in Table 2.

The rates available from these territories for 1961 and 1962 show a substantial further fall. For example, the 1961 and 1962 rates for Trinidad and Tobago were 44·9 and 40·0 respectively.

In some territories, infant mortality rates (1959–60) were considerably higher than those given in Table 2. Examples are shown in Table 3.

Table 2. *Fall in infant mortality rates in certain territories between* 1935 *and* 1960

	1935	1959–60
Barbados	220	60
British Guiana	122	57
Jamaica	137	51
Puerto Rico	127	44
Trinidad and Tobago	99	62
Surinam	82	44

Table 3. *Some high infant mortality rates*

	1959–60
Dominica	112
Grenada	79
Montserrat	135
St Lucia	98
St Vincent	138

In 1961 and 1962 there was a fall in some of these rates; thus, in Dominica the reported figures for these years were 93 and 74 respectively. But current rates remain high for these and certain other islands.

It is often suggested that, in the developing countries generally, the infant mortality rate is less closely associated with nutritional factors than the death rate in the 1–4 age group. Reasons given in support include: breast feeding tends to be maintained throughout the first 9–12 months of life and often longer, and up to this age largely fulfils the infant's nutritional needs; the infant grows well for 6–9 months, after which the growth curve 'flattens out'; the maximum incidence of serious malnutrition is between 1 and 3 years of age. This is *not* the situation in

the Caribbean where breast-feeding, as will be shown, is of relatively short duration. Table 1 and other facts given earlier show that a high mortality, unquestionably associated with malnutrition, occurs in the second half of the first year. There seems little doubt that malnutrition and its companion gastro-enteritis underlie, through their effects in the second 6 months of life, current high infant mortality rates in some territories, that they were largely responsible for the high rates prevailing in other territories until a few years ago, and that the fall in the latter has been due mainly to the partial prevention of these conditions. There is however, another important fact to be considered, namely the occurrence of malnutrition and gastro-enteritis in infants *below* the age of 6 months.

In Trinidad, Jelliffe, Symonds & Jelliffe (1960) studied a group of seventy children admitted to the paediatric unit in San Fernando General Hospital, South Trinidad, in 1958, with a diagnosis of malnutrition. Of these, sixty were below 12 months of age including twenty-seven aged 1–5 months. Their condition was ascribed primarily to the early replacement of the breast by insufficient and 'bacteriologically dangerous' artificial feeding. When I visited this unit in January 1964, the paediatrician in charge, Dr Isahak Mohammed, kindly gave me parallel figures for 1963; in that year there were sixty-five admissions with fifty-six children below 12 months including thirty-eight aged 1–5 months. The 1963 figures thus show the same preponderance of cases under 1 year and an even greater proportion under 6 months. Another point worth recording is that in the 1958 group there were eighteen cases of classical kwashiorkor and in the 1963 group only seven.

It is possible that the early occurrence of malnutrition, i.e. infants under 6 months, is reflected in the mortality statistics of some territories. In Barbados, the infant mortality rate fell from 135 to sixty between 1955 and 1960, and the age-specific mortality in the age group 1–4 from 8·1 to 3·5 in the same period. But it appears that the fall between 3 and 5 months is 'lagging'. Table 4, based on figures provided by PAHO, illustrates this point.

Table 4. *Deaths in Barbados, St Kitts and U.S.A.*—3 *months to* 2 *years* (1960–62)

(Age-specific death-rates.)

Age	Barbados	St Kitts	U.S.A.	Barbados/ U.S.A.	St Kitts/ U.S.A.
3–5 months	11·7	19·4	2·4	5·1	8·1
6–8 months	9·2	18·8	1·2	7·1	15·9
9–11 months	6·0	18·3	0·7	8·5	25·1
12–23 months	8·7	35·9	1·7	5·0	23·3

The significant point is that Barbados has less advantage over St Kitts in the age group 3–5 months than at ages over this period up to 2 years. This suggests that in Barbados there has been less success in preventing malnutrition in early infancy than in later infancy and the second year of life. Some support for this possibility is provided by data (unpublished) indicating a slackening in growth as early as the fourth month in babies brought to child health centres in Barbados.

Analysis of reported infant mortality by month in Trinidad might reveal similar trends. In Barbados and Trinidad the tendency towards early weaning seems to have proceeded further than in the Leeward and Windward Islands, which means very early cessation of breast-feeding and an extension of unsatisfactory and bacteriologically dangerous artificial feeding. Obviously, however, further studies are needed before conclusions can be reached. It would be necessary to reconcile the fall in total infant mortality in territories such as Barbados and Trinidad with an apparent tendency towards greater hazards to infants below the age of 6 months resulting from earlier weaning.

MORTALITY IN THE AGE GROUP 1–4

Mortality figures for this age group, which is a unit in vital statistics, are available for some Caribbean territories and for developing countries elsewhere. In the Caribbean generally fall in 'toddler' mortality has occurred in recent decades, coinciding with the fall in infant mortality. The figures shown in Table 5 for Puerto Rico, kindly supplied by the Health Department, are illustrative.

Table 5. *Mortality* (1–4) *in Puerto Rico*, 1940–60

	1940	1945	1950	1955	1960
Deaths in the 1–4 age group as percentage of total mortality	19·7	17·3	13·3	8·4	5·4
Deaths per 1000 children aged 1–4 (age-specific death-rate)	30·8	19·2	9·9	4·8	3·1

Between 1935 and 1959–60 the infant mortality rate in Puerto Rico fell from 127 to 44.

In Barbados and Trinidad (1959) the corresponding age-specific death-rates were about 3·5 and 3·1 respectively, which no doubt represents a large reduction on earlier figures. In Surinam, the reported rate dropped from about 5·2 in 1951–55 to 3·5 in 1961. The current U.S. rate is about 1·0.

It would no doubt be possible to break down the Puerto Rican figures given in Table 5 to show age-specific mortality for each year of life between 1 and 4, and the statistical records necessary for this purpose may be available in other territories, lying on the shelves of registrars' offices. I believe that such studies would show that in earlier years there was a high concentration of mortality in the age group 1–2, and that the fall which has occurred in mortality between 1 and 4 has been due primarily to fewer deaths between 1 and 2.

OTHER HEALTH STATISTICS

(*a*) *Deaths ascribed specifically to malnutrition*

In some territories, the mortality statistics include a number of deaths, nearly all in children below 2, under the following heads: 'malnutrition'; 'avitaminosis and other deficiency states'; 'deficiency states'; 'nutritional maladjustment'. All these seem to mean much the same thing. Such returns are useful in showing that serious problems of nutrition exist in the territory in question, but their

absence does not indicate the opposite. The criteria on which the diagnoses are made are inconsistent, varying in different territories and even in the same territory at different times. Hence such records are of limited value in assessing the nutritional situation and changes in it.

(*b*) *Broncho-pneumonia*

'Broncho-pneumonia' figures prominently in the vital statistics of the area as a cause of death in children, though it takes second place to gastro-enteritis. Uttley (1963), referring to Antigua, has written as follows about its association with malnutrition: 'Although the main killing diseases of early childhood are gastro-enteritis and broncho-pneumonia, these infectious conditions are normally terminal events, hiding from the physician the underlying state of malnutrition which greatly facilitates their lethal attack on the child and which is much harder to detect in their presence'. In the 1960 *Review of Health Services, British Guiana*, it is stated that many deaths in infants and young children are listed as gastro-enteritis and pneumonia, but are primarily due to malnutrition.

I found that medical and public health authorities in general shared these views on deaths from broncho-pneumonia. The significance of broncho-pneumonia is one of the many questions to which careful attention should be given in developing future public health and nutrition programmes in the Caribbean.

(*c*) *Measles*

According to a PAHO/WHO 'Summary of Four-Year Reports on Health Conditions in the Americas, 1957–60' (1962), measles is a serious and lethal disease of childhood in much of Central and South America. In Chile, Costa Rica, Ecuador, El Salvador, Guatemala, Hondurus, Mexico and Peru it ranked as the fifth to seventh cause of death, with the majority of deaths occurring in children under 4. This report states that the measles death-rates for five countries of Central America, and for South America, were 100 and 40 times respectively higher than in the United States. It also comments that 'in middle America, the measles death rate for children 1–4 years of age was higher than for infants' and introduces the idea of a relationship between fatal measles and protein-calorie malnutrition, suggesting that measles is more likely to kill a malnourished than a well-nourished child. This relationship is still open to question but, on the other hand, there is abundant evidence that measles often *precipitates* severe malnutrition. Thus, in a group of fifty-one children with kwashiorkor in a hospital in Peru, kwashiorkor followed measles in thirteen (Graham & Morales, 1963).

In West Africa measles is a formidable disease of childhood with a case mortality of about 5%, and is often followed by severe malnutrition; here also a relation between the severity of the disease and the state of nutrition of the child population has been suspected (Morley, Woodland & Martin, 1963).

Given these facts and speculations, it is interesting to note that measles scarcely figures at all as a cause of death in the vital statistics of Caribbean territories. In St Lucia, there was an epidemic of measles in 1957; 931 cases were reported, with six deaths, a low case mortality as compared with West Africa. Since then no

deaths from measles have been registered in this island. I asked medical and public health authorities in all the territories visited about measles and was told everywhere that the disease was common but not dangerous. This applies not only to the islands but also to Surinam and British Guiana on the South American mainland. It therefore appears that measles is not an important element in the malnutrition-disease complex in the Caribbean.

Study of the reasons underlying the apparently fortunate position of the area might throw light on the remarkable differences in the severity of measles in space and time.

MALNOURISHED CHILDREN IN HOSPITALS

In many territories the charts of a large proportion of infants and young children under treatment in hospital are labelled 'malnutrition'. These patients usually represent a concentration of cases from the whole or a considerable part of the territory concerned. I noted a general correspondence between the hospital picture in any given territory and the infant mortality rate and the state of development of maternal and child health services. The majority of the children admitted with a diagnosis of malnutrition were between the ages of 4 months and 2 years and were suffering from malnutrition of the marasmic type, associated with diarrhoea. I saw only a few cases of classical kwashiorkor, with oedema, dermatosis, enlarged liver and the characteristic appearance of misery. In the Caribbean kwashiorkor is less common than marasmus and the general impression of doctors is that it is seen less often to-day than 5–10 years ago. The children with kwashiorkor are somewhat older than those with marasmus, i.e. they are usually 15 months or over.

CHILD-FEEDING PRACTICES

The high incidence of malnutrition and gastro-enteritis in infants and young children is the result of child-feeding practices characteristic of the area. I tried to obtain as much information about these as possible in the different territories, mainly by questioning experienced doctors, health visitors and nurses. Visits to markets and shops provided useful information; for example, large displays of tinned milk products of many kinds, including expensive proprietory infant milk preparations, threw light on trends in child feeding, as did statements by shop-keepers such as 'we don't sell much arrowroot nowadays'. There is not much in the literature on the subject. Jelliffe (1955) described practices in Jamaica, but some changes have occurred since his account was written. The sketch which follows is based on these and other sources. While practices naturally differ to some extent from place to place, some generalizations can be made.

The duration of breast-feeding in the Caribbean is shorter than in Africa and Asia. Three to 6 months is usual and 9 months seems to be the limit except in a few communities, e.g. East Indians in British Guiana. This relates to the period during which the breast is offered to the child. A distinction must be made between breast-feeding as the sole or main source of the infant's nourishment, and partial breast-feeding which makes a smaller contribution to its needs. After 3 months or so, and often earlier, breast-feeding, if it does not cease altogether, becomes partial. The

mother may give the baby some breast milk before she leaves for work in the morning and again in the evening, and perhaps also during the night. The morning feed is often omitted. The mother usually seeks to continue partial breast-feeding as long as possible in the hope of avoiding pregnancy.

Throughout the area, this pattern of limited breast-feeding is changing in the direction of *less* breast milk. In some sophisticated urban circles the position now prevailing in countries such as England and the U.S.A. has been reached, i.e. artificial feeding is regarded as the norm and the breast as an out-dated organ for feeding purposes. This is an important fact, since such circles set the fashion. But more important is the general tendency towards a reduction of the infant's consumption of breast milk in the population as a whole, in response to economic and social pressures.

The foods given to supplement or replace breast milk include processed cow's milk, starchy roots and fruits and cereals. Among the processed milks, sweetened condensed milk, containing about 45% of sucrose, is popular because of its apparent cheapness; it was the first kind of tinned milk to be used in infant feeding in the Caribbean. Recently, however, its sale seems to have diminished, while the use in child feeding of the more expensive dried and evaporated whole milk products, and the still more expensive proprietory infant milk foods, has greatly increased. Simultaneously, there has been an extended use of dried skim milk which in terms of milk solids contains more protein than whole milk, but is much cheaper.

The main foods other than processed cow's milk are preparations of starchy roots and fruits such as sweet potatoes, yams, bananas, pumpkin and arrowroot (*Maranta arundinacea*), and various cereals. Arrowroot, which has a negligible protein content, took first place until a few years ago in some of the Leeward and Windward Islands, but is now less frequently used in that part of the Caribbean. In Trinidad, however, it remains a popular infant food, one of its uses being to 'strengthen' dilute milk solutions. It is also thought to be a good food for infants with diarrhoea. In the area generally, there seems to be a tendency to replace starchy roots and fruits by cereal preparations such as corn flour gruel and wheat flour porridge or gruel. Some of the familiar breakfast cereals, on sale everywhere, are being given to infants and young children; Quaker oats seems to be specially popular in several islands. Since cereal preparations are richer in protein than starchy roots and fruits, their growing use is a change in the right direction. In Jamaica some nutrition workers consider that the increased use of wheat flour gruel in infant feeding has helped to prevent malnutrition.

Fish and meat are rarely given to children under 18 months to 2 years; they are thought to produce worms. The same applies to beans, which are thought to be indigestible. Eggs are too scarce and expensive to be given to children in poor families. Where the influence of child health centres is making itself felt, fruit juice may be introduced into the infant's diet from about the third month.

The quantity of the diet received by infants and toddlers is as important as its quality. In the Caribbean as elsewhere supplementary foods are usually given in insufficient amounts during the weaning period and deficiency of calories and

deficiency of protein go together. Their inter-relationship is reflected in the terms 'protein-calorie deficiency' and 'protein-calorie malnutrition' now generally applied to the manifestations of malnutrition, in their various forms, in infants and toddlers in the developing countries.

The growing reliance on processed milk in infant and child feeding in the Caribbean is a trend which merits careful consideration. The same thing is happening in many tropical countries, though in few to the same extent as in the Caribbean. Processed milk provides protein of good quality otherwise lacking in the child's diet, based on pappy preparations of starch roots and fruits and cereals. If an infant is given less arrowroot and more tinned milk—even sweetened condensed milk—this represents an improvement. There is no possibility, in the area as a whole, of producing fresh milk in sufficient amounts to meet child feeding requirements. Objections to the increasing use of processed milk are made on two main grounds: first, its availability discourages breast-feeding and artificial feeding promotes gastro-enteritis and malnutrition; secondly, in its more expensive forms it is out of line with the purchasing power of poor families.

Breast-feeding unquestionably counters malnutrition and intestinal infection, so that its prolongation is most desirable. In child health centres in the Caribbean mothers are urged to avoid early weaning, and such exhortations should no doubt continue. But much experience shows the difficulty of extending the period of breast-feeding, and the strength of economic and social pressures in the opposite direction. As far as infant and child health is concerned, the encouragement of safe and satisfactory artificial feeding, in which processed milk largely replaces breast milk, seems of greater practical significance.

In cost, the proprietory infant milk foods head the list. These are skilfully advertised and enjoy social prestige because they are used by the well-to-do. They are extolled as 'the best food for infants', a claim often enhanced by the picture of the bonny baby on the tin. Even illiterate mothers, without access to the radio and newspapers, learn of their remarkable properties by hearsay. The result is that mothers insist on buying them, however deep a hole this makes in the family's resources, feeling that if they do not buy them they are failing in their duty to the child. But often they cannot buy them regularly, so that the expensive tin is made to last longer than it should by excessive dilution, and in effect the baby receives water coloured by the milk product. Further, the mother feels that she need not bother much about the rest of the infant's diet, so long as it is getting so valuable a food. Artificial feeding of this nature is not only deleterious in the nutritional sense; the necessary handling of the feeding bottle in insanitary homes is likely to cause infection. These infant foods are, of course, excellent in themselves. They are based on much nutrition research and when properly and fully used produce thriving infants. But undoubtedly they can be a menace to child health at a certain stage of economic and social development.

I noted that the giving of expensive infant milk foods evoked an emotional response on the part of nurses and doctors. Almost everywhere, it was the first thing I heard about on inquiring about infant feeding. Sometimes I was told that their sale should be prohibited. Among the reasons for these reactions is that

infants fed on highly diluted milk products will probably develop malnutrition and hence come to the attention of child health services and hospital doctors. In the population as a whole, however, their misuse may be less common than dramatic stories about it suggests, and may well be counterbalanced, to a growing extent, by their successful use in accordance with the instructions on the tin, by mothers who because of better wages can afford them easily. The actual situation is that the use of imported processed milk in infant and child feeding in the Caribbean is increasing every year, and is likely to go on increasing. In my view the child health services should accept this situation and adjust their activities accordingly. This means placing strong emphasis on the form of imported milk which is much the cheapest in terms of protein content, namely, dried skim milk.

COMMENT

Most of the facts I was able to gather about child malnutrition in the Caribbean during a tour which, though brief, offered an exceptional opportunity of visiting many territories, seemed to make sense when put together. Or perhaps it would be better to say that a rational pattern, needing completion by further studies, was discernible. In a general way, mortality statistics, morbidity records and the clinical picture of malnutrition can be related to child-feeding practices. For example, the age incidence of malnutrition and gastro-enteritis is explained by early weaning, partial or complete, and here the concept of 'weanling diarrhoea' adds clarification. The importance of malnutrition and gastro-enteritis in infants under 6 months needs, however, further investigation. Again, the reasons for the infrequency of classical kwashiorkor, and its regression during recent years, are not immediately obvious; possibly education in child feeding through maternal and child health centres and skim-milk distribution have reduced its occurrence in children at the most susceptible age, which is between 1 and 2 to 3 years.

The feeding practices themselves reflect economic and social factors, the availability of various foods, locally grown and imported, and the state of development of maternal and child health services. With regard to food supplies, the quantities of skim milk distributed are of special significance. The unusual structure of family life is an important factor.

This paper is confined to malnutrition in one age group, and of course attention must also be given to other sections of the population. In the longer report to the sponsoring organizations, malnutrition in age groups other than infants and toddlers was considered and the conclusion reached that this is, relatively speaking, of minor importance. Other sections of the report dealt with food supplies and the vital question whether reliance on imports can or should be reduced. The main purpose of the assignment was to recommend further studies which will give a more complete picture of the problem, and action to combat malnutrition. A brief account of the main recommendations relating to the most vulnerable groups will now be given.

INVESTIGATION AND ACTION

(*a*) There should be continuing study and analysis of the mortality statistics (current and past) of the different territories, with special reference to mortality in infants and young children and its relation to malnutrition. Attention should be given to the possible influence of malnutrition on mortality in children under 6 months of age, and to trends in mortality from certain causes, e.g. gastro-enteritis and broncho-pneumonia. An associated question is the ascribing of deaths to 'malnutrition', 'deficiency states', 'avitaminosis', etc.; here clarification and the 'standardization' of diagnoses are needed. The same applies to morbidity data on malnutrition. Studies of vital statistics should not ignore hospital admission figures.

Good opportunities exist for such investigations in the Caribbean. In general, small 'manageable' island populations are involved and vital statistics are more accurate than in most developing countries. Further, medical and public health services have been in operation for a considerable period of time, which makes possible illuminating retrospective studies. Facts with a bearing on the causes of infant and child mortality elsewhere in the tropics would probably be elicited. In the Caribbean itself trends in vital statistics are an important indication on the effectiveness of measures for preventing malnutrition.

(*b*) Further studies should be made of child-feeding practices in the different territories.

The outline given in this paper needs filling in. Differences in feeding practices in different territories could be correlated with the prevalence of malnutrition. A watch should be kept on changing trends.

(*c*) More information is required about the social and economic background of individual children suffering from malnutrition and of the feeding errors responsible for their condition.

When visiting wards in which malnourished children were under treatment, I often asked the questions: 'Who are these children? Why are they here?' Various answers were given. There were impressions that many of them came late in a closely spaced sequence of births in a large family, and quite contrary impressions that they were usually the children of very young mothers. Sometimes poverty was emphasized, sometimes the lack of maternal care, and sometimes the connexion between malnutrition and 'shanty towns'. With regard to maternal care, diversion of attention from the child because another was on the way, and its being taken over by the grandmother, were sometimes mentioned. On the dietary side, abrupt weaning and the misuse of expensive milk products were among the reasons given for the children's condition.

Systematic investigation of this aspect of the problem is recommended. It would not be difficult. I visited child health centres with well-kept records where a few days' analysis of record cards would provide some of the answers. The Infant Jesus Malnutrition Hospital in Roseau in Dominica kindly produced for me overnight some data on the last twenty-five admissions, showing the marital and working status of the parents of these children and whether the father was providing support or not.

(*d*) The treatment of children with malnutrition, excellent in a few hospitals, is unsatisfactory in the majority. The children do not receive enough protein and calories, and nursing care is inadequate. Stay in hospital is prolonged and relapse often occurs after discharge. Steps to improve, and even to standardize to some extent, methods of treatment throughout the area are therefore recommended. PAHO has already made a beginning in this direction. The satisfactory rehabilitation of children after severe malnutrition is an associated question. It can be promoted by good hospital treatment, the careful instruction of mothers on feeding methods, and the following up of convalescent children by maternal and child health and other services. The establishment of 'rehabilitation centres', to which the child could be sent after reaching a certain stage of recovery, has been proposed. This idea merits examination, but it is likely that improved treatment and subsequent supervision would make such centres unnecessary.

(*e*) Maternal and child health services and centres have primary responsibility for combating malnutrition, through education and the distribution of supplementary foods. In some territories they are well organized and reach a large percentage of the population; in others their coverage is limited and trained staff inadequate. A realistic study of the state of development of maternal and child health services in the area is therefore recommended. A close relation would unquestionably be found between this and the prevalence of malnutrition. It is well known, for example, that the remarkable fall in infant and toddler mortality in Barbados in recent years has been associated with the creation of efficient maternal and child health services by an outstanding Director of Medical Services.

As part of this study, the content of teaching on child feeding through maternal and child health and other services should be examined, with the object of revision if necessary. Present teaching sometimes reflects too closely the text-books of the developed countries and needs more adaptation to local conditions.

(*f*) During recent years skim milk has been distributed in large quantities, mainly through maternal and child health services. The amount distributed per head of population has probably been larger in the Caribbean than anywhere else in the world. Most doctors and nurses are convinced that skim milk has done much to prevent malnutrition, and that its use should be continued and extended. I believe their views are fully justified. Certain studies on skim milk are recommended in the report. An attempt should be made to compare the quantities distributed with trends in the prevalence of malnutrition. Methods of distribution, less efficient in some territories than others, could be improved by elementary studies to discover the best procedures. It is desirable that the commercial sale of skim milk powder should be encouraged, to supplement or replace free distribution. Among the ways of doing this is to pack the skim milk in attractive containers. Here again some simple investigations and trials are needed.

Studies and activities of these kinds, and others concerned with food supply and consumption, agriculture, growth records, the prevalence of anaemia, etc., would be facilitated, and more rapid progress would be made in preventing malnutrition, by establishing a Caribbean Nutrition Centre. A final recommendation to PAHO/WHO and FAO deals with the functions and structure of such a centre.

DISCUSSION

The conventional practice of grouping deaths in the periods 0–1 and 1–4 has, in the Caribbean and other developing areas, largely obscured the peak in mortality associated with weaning, and retarded recognition of one of the major causes of death in man, namely the combination of malnutrition and gastro-enteritis in infants and toddlers wholly or partially removed from the breast. It is only when the mortality records are broken down into shorter intervals, as in Table 1, that the peak becomes evident. In the developing countries its timing turns on local breast-feeding practices; when, as in much of the Caribbean, weaning takes place between 3 and 6 months, it is found between 6 months and 2 years. If weaning is even earlier, as seems to happen in Trinidad, a high mortality associated with 'weanling diarrhoea' occurs below 6 months, mainly from the third month onward. When breast feeding is prolonged for 9 months or more, the major incidence of sickness and mortality due to malnutrition and associated conditions is between 1 and 3 (but probably diminishing between 2 and 3). Mortality statistics, past, present and future, could advantageously be viewed in the light of these facts. A change in, or rather the extension of, methods of presenting mortality statistics in public health reports from the developing countries would bring out the importance of the malnutrition-disease complex as a cause of death. For this purpose, mortality from 6 to 12 months, and from 1 to 2 or 3 years of age, would be more informative than the conventional infant mortality rate and mortality between 1 and 4. When weaning is exceptionally early, mortality from 3 to 5 months should receive special attention.

The WHO 'Expert Committee on Medical Assessment of Nutritional Status' (1963), in discussing vital statistics and malnutrition, comments that: 'there is much to be said for analysing mortality statistics according to age intervals smaller than those usually employed. In much of the world grouping deaths of children 1 to 2 years old would probably be found most useful in assessing mortality from protein-calorie malnutrition. Further inquiries may reveal other age-specific mortality rates significant in relation to malnutrition.' These comments are relevant to the Carribbean.

Malnutrition in infants and young children in the Caribbean could be greatly reduced or even eliminated in a few years by suitable measures, in spite of any difficulties created by the unusual pattern of family life. Real progress in this direction is already being made, evidenced, for example, by infant mortality statistics (Table 2). But such an achievement would increase food requirements through acceleration of population growth. The whole problem of food supplies in the area, particularly its growing dependence on imports, has not been considered in this paper, though discussed in my report to PAHO/WHO and FAO. The Caribbean Nutrition Centre, if established, will be concerned with these basic questions as well as with the prevention of malnutrition.

SUMMARY

1. In many parts of the Caribbean there is a high mortality between 6 months and 2 years due to malnutrition and gastro-enteritis. These two conditions are so closely inter-connected that they can conveniently be regarded as a single syndrome, for which the term 'weanling diarrhoea' has recently been suggested. The clinical picture revealed by visits to children's wards was in line with the vital statistics.

2. The prevalence of malnutrition and gastro-enteritis in infants and young children is the result of child-feeding practices characteristic of the area. The duration of breast-feeding is shorter than in Africa and Asia. After 3 months or so breast-feeding, if it does not cease altogether, becomes partial, and in general there is a steady change in the direction of *less* breast milk. In some territories weaning at an even earlier stage in infancy seems to result in the common occurrence of malnutrition in infants under 6 months of age.

3. The foods given to supplement or replace breast milk include processed cow's milk, starch roots and fruits and cereals. Imported processed milk supplies good quality protein otherwise lacking in the child's diet, but the use of expensive proprietory infant milk foods, out of line with family purchasing power and given in over-diluted form, is among the causes of malnutrition. The use in infant and child feeding of dried skim milk—much the cheapest kind of milk in terms of nutritive value—is increasing and this trend should be encouraged.

4. Most of the malnutrition is of the 'marasmic' type. Classical kwashiorkor, now comparatively rare, was seen more often 5–10 years ago.

5. It is suggested that the conventional practice of grouping deaths in the periods 0 to 1 and 1 to 4 has, in the Caribbean and other developing areas, retarded recognition of the importance of malnutrition and gastro-enteritis as the principal cause of death during and after the weaning period. The analysis of mortality statistics according to shorter age intervals is desirable.

6. Further studies and action are recommended concerned with the following: the continuing analysis of mortality and morbidity statistics to elicit relationships with malnutrition; child feeding practices in the different territories; the social and economic background of individual victims of malnutrition and of the feeding errors responsible for their condition; improvement in the treatment of malnourished children; the scope and efficiency of maternal and child health services; the distribution of dried skim milk and its extension.

7. The establishment of a Caribbean Nutrition Centre will contribute to the prevention of malnutrition and help in solving long-term problems of food supply and nutrition in the area.

I must gratefully acknowledge the help I received during the assignment from United Nations staff members and from government departments and individuals in the various territories, where suitable programmes of visits were arranged and relevant information provided. Thanks are also due for kind personal hospitality which helped to make a tour in one of the most beautiful and interesting parts of the world an unforgettable experience.

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Viruses in acute respiratory infection in a general community†

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INTRODUCTION

The renaissance of tissue-culture techniques a decade ago led to the discovery of many viruses which cause acute respiratory infections. Investigations conducted to assess the role of these viruses mostly involved conveniently accessible groups such as children in hospital and residential institutions, students, and military recruits. These groups consisted of young persons living in socially similar conditions; the turnover in these populations often being rapid. Such conditions favoured the introduction and spread of infection. These studies did not provide information as to the relative importance of different viruses causing acute respiratory infection in normal civilian communities. Comparatively little is known of the aetiology of acute respiratory infections in members of the community living in their own homes. We have, therefore, studied the role of viruses in acute respiratory infections from September 1962 to August 1963 in two general practices in Cambridge.

Parainfluenza infections have been considered in detail in a previous communication (Banatvala, Anderson & Reiss, 1964), and the findings of infection with influenza virus and Eaton agent (*Mycoplasma pneumoniae*) will be presented later. This paper presents the overall results of our survey.

STUDY POPULATION

The study population was selected to provide a representative sample of the permanent community of Cambridge, i.e. the population apart from students. The population consisted of the patients of two general practitioners (T.B.A., B.B.R.) working in different parts of Cambridge, with a combined total of 5178 patients. A chi squared (χ^2) test was done in order to determine whether the study population was representative of the community of Cambridge. The age and sex structure of the combined practices was compared to that of Cambridge City. The two populations were divided into the following age groups: 0–4, 5–14, 15–39, 40–59, 60 and over. Females in the study population provided a reasonable sample of the

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community ($\chi_4^2 = 7{\cdot}95$, not significant at the 5% level), but there were fewer males aged 0–4 than expected in the study population ($\chi_4^2 = 36{\cdot}1$, significant at the 0·1% level). Apart from the 0–4 age group, the male study population was found to be reasonably representative of the community of Cambridge ($\chi_3^2 = 2{\cdot}82$, not significant at the 5% level).

SELECTION AND RECORDING OF CASES

Details on all patients who needed to consult their general practitioner with an acute febrile respiratory infection either at home or in the consulting room were recorded on standard cards. One of the following clinical diagnoses was made on each patient:

1. Influenza.
2. Common cold.
3. Primary atypical pneumonia (PAP).
4. Acute respiratory disease (ARD).
5. Acute pharyngitis/tonsillitis.

ARD was further subdivided into the following groups:

a. Obstructive laryngo-tracheo-bronchitis of infants (croup).
b. Acute epidemic bronchiolitis of infants.
c. Acute pneumonitis of infants.
d. Pharyngo-conjunctival fever.
e. Epidemic kerato-conjunctivitis.

This classification is a modification of that devised by Stuart-Harris (1953). Patients seen within 72 hr. from the onset of symptoms and who consented to have specimens taken were immediately referred by the general practitioner for laboratory investigation.

METHODS

Collection of specimens

On notification of a case by the general practitioner, one of us (J.E.B.) would visit the patient at home and collect epidemiological data together with specimens for virological investigation. These consisted of:

(1) A pharyngeal swab. This was immersed in 4–5 ml. of medium 199 containing penicillin 500 units/ml., streptomycin 500 μg./ml., and amphotericin B 8μg./ml. It was transported to the laboratory on crushed ice.

(2) Blood, acute phase sample (10 ml.).

(3) Blood, convalescent phase sample (10 ml.). This was taken 2–3 weeks after the acute phase sample was collected. At this time additional information as to the course of the illness and spread of the infection within the family was obtained. Blood was not taken from infants and young children.

Pharyngeal swab fluid (0·2 ml.) was inoculated into secondary monkey kidney and HEp-2 cell cultures within 2 hr. of collection. Bacterial isolations were only attempted in patients with acute tonsillitis/pharyngitis. Both bacteriological and virological studies were made in a proportion of these cases in order to determine whether viruses had any role in the clinical picture of acute tonsillitis/pharyngitis.

Tissue culture

Monkey kidney. Monolayers were grown in medium 199 containing calf serum 5 %, penicillin 100 units/ml., streptomycin 100 μg./ml., amphotericin B 1·6 μg./ml., SV 5 antiserum 0·02 % and sodium bicarbonate 0·088 %. Maintenance medium was similar to growth medium, except that no calf serum was added. Cultures were thoroughly washed to remove all traces of calf serum before inoculation.

HEp-2. Tissue culture monolayers were grown in Eagle's medium containing 10 % calf serum and 5 % human serum. Antibiotics were added as for monkey kidney cultures. Maintenance consisted of Eagle's medium, antibiotics, 1 % calf serum and 0·088 % sodium bicarbonate. Cultures were washed to remove all traces of human serum before inoculation. Both monkey kidney and HEp-2 cultures were incubated in stationary racks at 35° C.

Fertile hens' eggs. When influenza was prevalent, specimens were inoculated into the amniotic sac of 11-day-old fertile hens' eggs. The eggs were incubated for 72 hr. at 35° C., after which the aminotic fluid was tested for haemagglutination.

Identification of viruses

Tissue cultures were inspected daily for cytopathic effect (C.P.E.). Fluids from cultures showing C.P.E. were passed into fresh cultures and the virus identified by neutralization tests using specific antisera. Monkey-kidney cultures were tested on the third, fifth and tenth days of incubation for haemadsorption (Vogel & Shelokov, 1957). Fluid from cultures exhibiting haemadsorption together with amniotic fluid exhibiting haemagglutination was passed into fresh monkey-kidney cultures and the virus identified by haemadsorption inhibition tests using rabbit antisera to parainfluenza 1, 2, 3 and SV 5 viruses, and ferret antiserum to influenza A 2 virus according to the method described by Chanock *et al.* (1958).

Serology

Complement-fixation tests (CFT)

CFT's were carried out using Perspex plates employing overnight fixation at 4° C. as described by Bradstreet & Taylor (1962). Sera were tested against the following antigens: influenza A, B and C, parainfluenza 1, 2, 3 and sendai virus, respiratory syncytial virus (RS virus), adenovirus, psittacosis and R. burneti. CFT's employing Eaton agent antigen were performed on the sera of patients with PAP or on family contacts of such cases who later developed acute respiratory infections. These tests were carried out by Dr B. P. Marmion (Public Health Laboratory, Leeds), using the method described by Goodburn, Marmion and Kendall (1963).

Haemagglutination-inhibition tests (HI)

Before testing, sera were treated with cholera vibrio extract and then inactivated at 56° C. in order to remove non-specific inhibitors. HI tests for influenza (A 2 Sing. 57) were performed on all patients during the time that influenza was prevalent, according to the method described by the W.H.O. (1953). During the time

that parainfluenza viruses were prevalent, HI tests for parainfluenza 1, 2 and 3 were performed by Dr R. B. Heath (St Bartholomew's Hospital, London) according to the method described by Heath, Tyrrell & Peto (1962).

Neutralization tests

Neutralization tests were performed on sera from all patients during the time that influenza was prevalent using fragments of chorioallantoic membrane on egg pieces as described by Fazekas de St Groth, Withell & Lafferty (1958). The same strain of virus was used as in the HI tests. In all these tests a fourfold or greater increase in antibody titre was regarded as significant.

RESULTS

Incidence

During the investigation there were 592 spells of acute respiratory infection of sufficient severity to require medical attention; this represents an incidence of 11·4 spells per 100 persons. The age and sex specific incidence rates are shown in Table 1. Pre-school children (ages 0–4) had the highest incidence rates (51·6 spells per 100 persons), males having a slightly higher rate than females. The incidence rates decreased in successive age groups.

Table 1. *Age and sex specific incidence rates (spells) per 100 persons*

Age (years)	No. at risk		Attack rate (spells) per 100 persons		
	M	F	M	F	M and F
0–4	145	177	55·2	46·8	51·6
5–14	364	323	25·8	23·5	24·5
15–39	977	872	5·6	7·7	6·6
40–59	693	669	6·1	6·4	6·2
60+	420	538	5·5	5·4	5·4
All ages	2599	2579	11·3	11·6	11·4

Seasonal distribution of cases

The weekly distribution of respiratory infections occurring in the combined practices are compared with the number of new claims to sickness benefit in Cambridge (all causes) in Fig. 1. Laboratory studies confirmed that the sudden increase in cases recorded by the general practitioners in February and March 1963 were due to influenza A (Asian strain). This corresponded to the increase in new claims for National Insurance sickness benefit in Cambridge. The monthly distribution of: (*a*) new claims to sickness benefit, (*b*) cases occurring in the two practices, (*c*) cases referred for laboratory investigation, are shown in Fig. 2. All three curves show similar trends, suggesting a correlation between cases investigated in the laboratory, cases occurring in the combined practices and those occurring in the insured population of Cambridge.

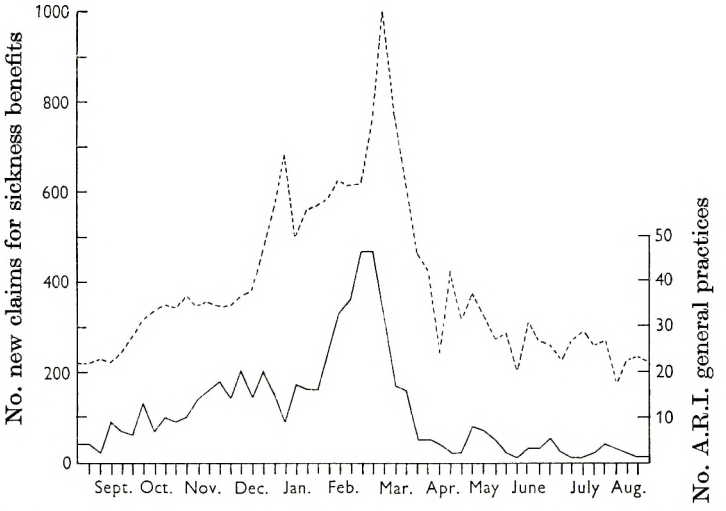

Fig. 1. Weekly distribution of new claims for sickness benefit, and incidence of acute respiratory infection in two general practices. ...., Sickness absence claims; —, general practice records.

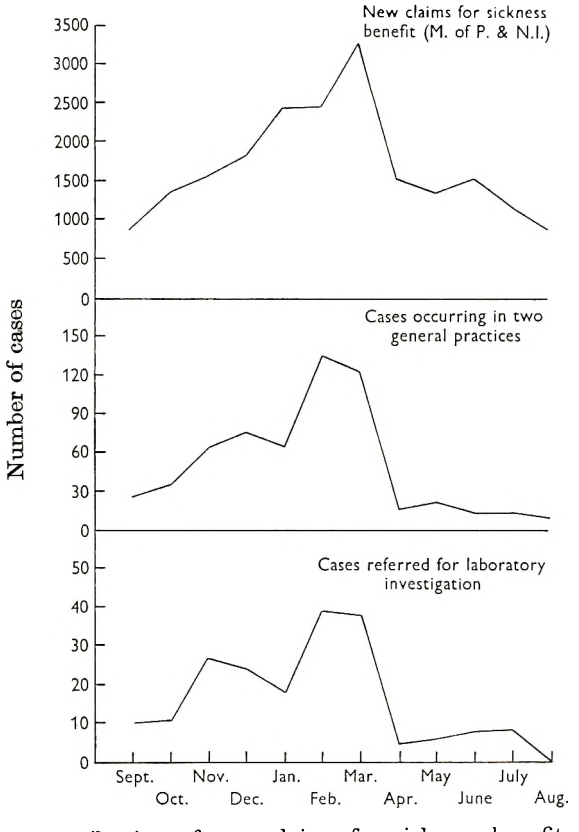

Fig. 2. Monthly distribution of new claims for sickness benefit, acute cases of respiratory infection in two general practices, and cases referred for laboratory investigation.

Results of laboratory investigations

From the 592 spells of acute respiratory infection occurring in the combined practices, 195 were investigated in the laboratory (33 %). Diagnosis in adults was established by isolating a virus and demonstrating concomitant significant sero-

Table 2. *Summary of principal laboratory findings*

Aetiological agent	Isolation* alone	Isolation and serology	Serology alone	Total
Influenza A	11	18	27	56
Parainfluenza 1	17	3	7	27
Parainfluenza 2	2	—	—	2
Parainfluenza 3	2	—	4	6
Adenovirus	1	1	2	4
Respiratory syncytial virus	—	—	3	3
Eaton agent	—	—	9	9
β-Haemolytic streptococcus	15	—	—	15
All agents	48	22	52	122

* Blood was not taken from young children; diagnosis being by virus isolation alone.

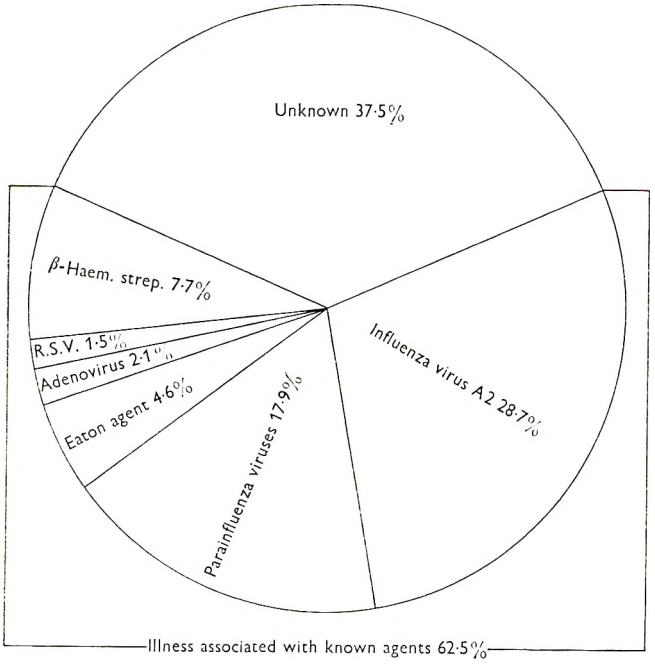

Fig. 3. Distribution of aetiological agents associated with acute respiratory infections in the combined practices.

logical evidence of infection, or by serological evidence alone. Only two adults were reluctant to give blood; diagnosis in these cases was by virus isolation alone. However, particularly in influenza and parainfluenza infection in children, secondary adult cases frequently occurred in the same family. It was often

possible to isolate from them a similar virus and demonstrate a significant rise in antibody titre to that particular virus antigen.

Table 2 summarizes the laboratory results. An aetiological agent was detected in 122 of the 195 cases investigated (62·5 %). Fig. 3 shows the proportion of infections associated with different aetiological agents. Viruses (i.e. excluding Eaton agent and streptococcal infections) were responsible for 98 of the 195 laboratory investigated cases (50·3 %). Myxovirus infections, i.e. influenza and parainfluenza infections, featured prominently, being responsible between them for 46·6 % of all infections investigated.

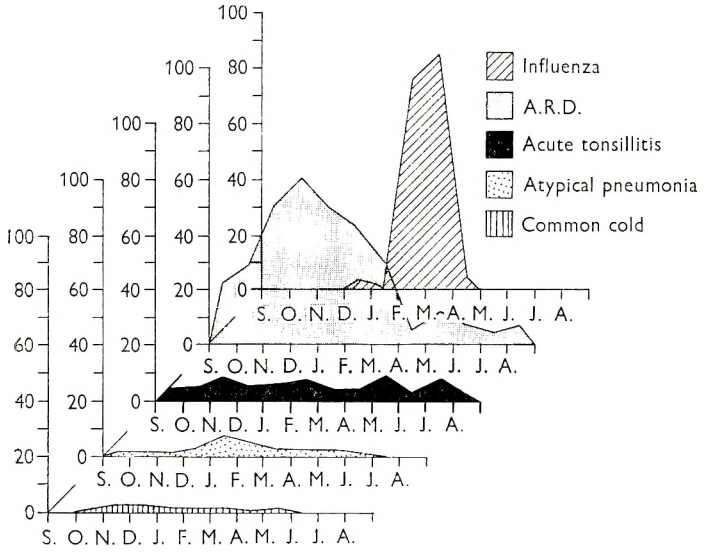

Fig. 4. Monthly distribution of clinical diagnostic categories.

Association between laboratory and clinical diagnosis

Study of the monthly distribution of clinical diagnostic categories (Fig. 4), together with the monthly distribution of aetiological agents (Fig. 5), establishes a link between the clinical and laboratory diagnosis. There were few clinical cases of influenza before February and after April 1963. Cases initially diagnosed as influenza at other times were mostly due to viruses other than influenza, such as the parainfluenza viruses. However, between February and April 1963, a clinical diagnosis of influenza was frequently confirmed by laboratory studies. Thus, from 165 cases of clinical influenza occurring between February and April 1963, sixty-three were referred for laboratory investigation. Evidence of influenza A (Asian strain) was obtained in fifty-six of these patients (89 %). The serological findings are summarized in Table 3. A significant increase in antibody titre by CFT was detected in forty-one of forty-five paired sera tested (91 %), whereas neutralization tests showed a significant rise in twenty-three of forty-two paired sera tested (55 %). HI tests were the least effective, a significant rise occurring in only fifteen of forty-five paired sera tested (33 %). HI and neutralization tests between them revealed only four cases of influenza which were not detected by CFT. During

the influenza epidemic, the convalescent phase sample of blood was usually collected 10–14 days after the acute phase sample. It has been shown (Jensen, 1961) that HI antibodies tend to develop later than CF antibodies. Had the convalescent sample of blood been collected at the end of the third week, then more significant rises by HI and neutralization tests might have been shown.

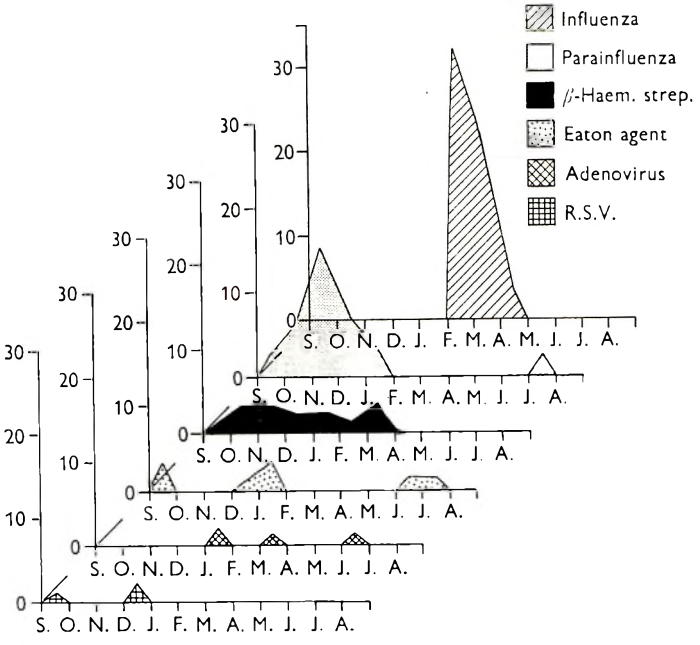

Fig. 5. Monthly distribution of aetiological agents in cases referred for laboratory investigation.

Table 3. *Summary of serological findings in influenza infection*

Significant rise by...	CFT, HI and neut.	CFT and neut.	CFT and HI	CFT only	Neut. and HI	Neut. only	HI only	Total
No. of cases	10	9	4	18	1	3	0	45

CFT = Complement-fixation test; Neut. = neutralization test; HI = haemagglutinin-inhibition test.

Acute respiratory disease was prevalent throughout the year, particularly during winter months. Much infection in this category was due to infection by parainfluenza viruses, particularly parainfluenza 1. During the 5 months ending 31 January 1963, parainfluenza viruses were responsible for 40% of all acute respiratory infections investigated. There were few cases of parainfluenza infection after January, influenza then becoming epidemic. Of thirty-five patients with parainfluenza infection, twenty-one were children most of whom had acute laryngo-tracheo-bronchitis (croup) of varying severity. Evidence of parainfluenza infection was obtained from fourteen adults many of whom had an influenza-like illness.

A diagnosis of PAP was made in twenty-five patients, cases occurring throughout the year. Evidence of Eaton agent infection was obtained from seven of ten cases referred for laboratory investigation (70 %). A cold agglutinin screen test was employed at the bed-side according to the method described by Garrow (1958) and was positive on four occasions (57 %). In addition, evidence of Eaton agent infection was obtained in two patients who were family contacts of patients with PAP, both of whom developed an influenza-like illness without pneumonia. Evidence of adenovirus infection occurred infrequently (four cases). An isolation was made on two occasions (type 2 and type 6). Three patients had ARD (pharyngo-conjunctival fever), whilst the other had an influenza-like illness.

There were three patients with RS virus infections (two adults and one child aged 14 months). One of these adults was the child's aunt, who developed an acute respiratory infection with a sore throat, cough and hoarse voice. The child subsequently developed acute laryngo-tracheo-bronchitis from which he died in hospital.

Since only febrile respiratory infections sufficiently severe to require medical attention were included in this investigaton, few cases of common cold were recorded. We did not attempt to isolate rhinoviruses.

Throat swabs for bacteriologial investigation were taken from fifty patients with acute tonsillitis and group A streptococci were isolated from thirty-two of these specimens (64 %). Of seventeen cases submitted for combined virological and bacteriological investigation, group A streptococci were isolated from fifteen, but no viruses were detected.

DISCUSSION

Surveys in general practice have shown that acute respiratory infections are a leading cause of morbidity in general communities, accounting for between 30 and 35 % of all consultations (Pemberton, 1949; Fry, 1957; Davies, 1958; Logan & Cushion, 1958). Recent technical developments have made possible the identification of many viruses responsible for these infections. Nevertheless, few studies incorporating such techniques have been performed on general communities. The regular collection of fresh specimens, the necessity of prompt inoculation into tissue culture and the collection of paired sera present difficulties in surveys on non-institutional communities.

Robinson *et al.* (1962), in a survey on a general population in Atlanta, Georgia, established a diagnosis in 26·4 % of cases of acute respiratory infection. As in Cambridge, parainfluenza infection featured prominently, being responsible for 14 % of all infections investigated. Higgins, Ellis & Boston (1963), in a general practice survey in Cirencester, identified an aetiological agent in 23·4 % of respiratory infections, but performed serological studies in only a limited number of cases. Clark *et al.* (1964), in a survey conducted on children admitted to hospital, together with a few patients with acute respiratory infection who consulted their general practitioner, established a diagnosis in 41 % of their cases. As in Cambridge, influenza and parainfluenza viruses featured prominently.

Woodall, Rowson & McDonald (1958), in a general practice survey during the 1957 epidemic of Asian influenza, estimated that 31 % of their patients developed

influenza. By contrast, in 1963 in Cambridge, although the volume of the general practitioner's work was considerably increased when influenza was epidemic, only 3·2% of patients developed influenza. Asian influenza, in 1963, was probably performing a 'mopping up' operation, attacking remaining susceptible persons who had escaped infection in 1957–8 and 1958–9, years in which the Asian influenza virus caused major epidemics.

There were few cases of adenovirus infection, for although these viruses are prominent causes of acute respiratory infection in military populations (Woolridge *et al.* 1956; Hilleman *et al.* 1957; van der Veen & Dijkman, 1962), and in children living in closed communities (Kendall *et al.* 1957; Bell *et al.* 1961), studies on civilian communities have revealed that adenovirus infection is responsible for only a small proportion of acute respiratory infections (Jordan *et al.* 1956; Grieble *et al.* 1958; Robinson *et al.* 1962; Higgins *et al.* 1963).

There were few cases of RS virus infection in the combined practices during 1962–63. RS virus is frequently associated with lower respiratory infection in infants and young children, particularly with bronchiolitis and pneumonia (Beem *et al.* 1960; Lewis, Rae, Lehmann & Ferris, 1961; Andrew & Gardner, 1963). Infection tends to occur in well-defined outbreaks, and it is probable that 1962–63 represented a year in which these viruses were not prevalent in Cambridge.

Since bacteriological investigations were only performed on patients with acute tonsillitis/pharyngitis, it is not possible to assess the role of pathogenic bacteria as a cause of other types of respiratory infection seen in this survey. However, other investigations have revealed that, apart from acute tonsillitis, pathogenic bacteria play a relatively small role in the aetiology of acute respiratory infections. Dingle *et al.* (1953) concluded from a study of 4428 acute respiratory infections over a two-year period in Cleveland, Ohio, that only 2·5% of infections were of bacterial origin. Higgins *et al.* (1963), in their general practice investigation, found β-haemolytic streptococci associated with 3·5% of acute respiratory infections, whilst Hilleman *et al.* (1962), in a study of respiratory infections in 677 children in hospital and outpatient clinics, together with a group of 155 industrial workers, found that there was no evidence of primary bacteriological cause in any of their cases.

In Cambridge an aetiological agent was not detected in 37·5% of cases investigated. Had blood been taken from young children and techniques for the identification of rhinoviruses adopted, it is probable that more laboratory diagnoses would have been made. Rhinoviruses typically cause common colds (Hobson & Schild, 1960; Hamre & Procknow, 1961), and therefore would be unlikely to be encountered frequently in our survey since only febrile infections requiring medical attention were studied. However, there is some evidence that rhinoviruses may cause more severe illness in young children (Reilly *et al.* 1962). In addition, evidence of Eaton agent infection was sought only in patients with PAP or their contacts in this survey. Chanock *et al.* (1961), in an outbreak of respiratory infection in a recruit training camp, demonstrated that Eaton agent caused upper respiratory and inapparent infections more commonly than pneumonia. The role of Eaton agent infections in upper respiratory infections in civilian communities

has yet to be established. One may, finally, speculate as to whether some of the infections in which no aetiological agent was detected were due to agents which remain to be discovered.

This survey was conducted over a limited period and only a relatively small number of cases were studied. One cannot be certain that at other times and in other places similar findings will occur. There is a clear indication for more prolonged observation employing the newer techniques for the identification of such agents as the rhinoviruses and Eaton agent so that the role of these agents in mild as well as severe infections can be assessed.

SUMMARY

The aetiology of acute respiratory infections between September 1962 and August 1963 was studied in two general practices in Cambridge. These practices were reasonably representative of the permanent community of Cambridge.

There were 592 spells of acute respiratory infection in the combined practices, representing an incidence of 11·4 spells per 100 persons. Children aged 0–4 had the highest rates (51·6 spells per 100 persons).

It was possible to establish a diagnosis in 62·5 % of cases investigated. Influenza and parainfluenza infections featured prominently, being responsible between them for 46·6 % of all respiratory infections investigated. From September 1962 to January 1963, parainfluenza viruses were prevalent, causing acute laryngo-tracheo-bronchitis in children (croup), and an influenza-like illness in adults. From February to April 1963, influenza A (Asian) was epidemic, a clinical diagnosis of influenza being frequently confirmed by laboratory studies at this time. There were nine cases of Eaton agent infection, seven of which had PAP, the other two being family contacts who later developed influenza-like illnesses.

Adenovirus (four cases), and RS virus (three cases) were not prevalent to any large extent in Cambridge during the survey.

We are indebted to many for their contributions to this study: Dr A. P. Waterson for his help and encouragement throughout this investigation; Mrs I. Nitkin, for her technical assistance; Miss E. R. Dalton, Department of Human Ecology, Cambridge, for statistical advice, Dr C. M. P. Bradstreet, Central Public Health Laboratory, Colindale, for supplying many diagnostic reagents; Dr G. Swan and Dr R. Bangham for clinical assistance; Dr C. G. Eastwood, M.O.H., City of Cambridge, for providing National Insurance sickness absence claims. The National Polio Research Fund gave generous support and enabled this investigation to be made.

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