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The egg yolk reaction of *Staphylococcus aureus* isolated from burns

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A large majority of strains of *Staphylococcus aureus* were found by Gillespie & Alder (1952) to produce opacity when grown in egg yolk broth, apparently through the action of a lipase on certain triglycerides. Of the strains isolated from out-patients, 82 % were 'egg yolk positive' (EY^+), but only 45 % of those isolated from in-patients gave a positive reaction; there was a strong association between penicillin-resistance and a negative egg yolk (EY^-) reaction. Parker (1958) confirmed the association of negative egg yolk reaction with penicillin resistance, and found that such strains were particularly common in superficial lesions, including wounds; strains which caused deep lesions usually showed a positive egg yolk reaction.

We have tested strains of *Staph. aureus* isolated from burns in three periods (1958, 1960 and 1962) and from several other sources for egg yolk reaction; the association of this reaction with some other features, including phage type, antibiotic sensitivity and mercuric chloride resistance (Moore, 1960) has been examined. The relevance of these data to the epidemiology of staphylococcal infection is discussed.

MATERIALS AND METHODS

Tests for egg yolk reaction

A liquid egg yolk medium was prepared in the manner described by Gillespie & Alder (1952).

To facilitate the examination of large numbers of strains, a solid egg yolk medium was developed by one of the authors (B.J.C.); similar media have been described by Colbeck (1956), Carter (1960), Innes (1960) and Baird-Parker (1962). The formula which was found to give optimal results in tests which will be described separately (Collins, to be published) was yeastrel agar containing 5 % egg yolk and 5 % horse serum. Yeastrel agar contained the following ingredients: Yeastrel, 3 g.; peptone (Oxoid), 5 g.; sodium chloride, 5 g.; sodium dihydrogen phosphate, 3 g.; agar (Oxoid No. 3), 8 g.; and distilled water, 1000 ml. The mixture was steamed, filtered, adjusted to pH 7·4–7·6 and autoclaved at 121° C. for 15 min. To 18 ml. amounts of this medium cooled to approximately 50° C. were added 1 ml. of horse serum and 1 ml. of egg yolk (obtained aseptically from fresh farm eggs). The medium was poured into sterile plastic Petri dishes, 10 ml. per plate.

Plates of egg yolk agar were spot-inoculated with *Staph. aureus*, 16 strains per

plate, from overnight infusion broth cultures; a standard platinum loop was used. After overnight incubation at 37° C., growth of EY+ strains was surrounded by an opalescent zone of surface precipitation, about 0·5 mm. wide, surrounded by a sharply demarcated halo of clearing in the turbid medium, extending from 1 to 5 mm. from the edge of the disk of growth; the opalescent precipitate was absent when zones of clearing were narrow. EY− strains showed neither the opalescent precipitate nor the halo of clearing. About 98 % of a series of 825 strains gave the same results on solid egg yolk medium examined after overnight incubation and in liquid egg yolk medium examined, in the manner described by Gillespie & Alder (1952), after 3 days' incubation; a small proportion of strains gave a weak egg yolk reaction. After this comparison of the two media further tests were made only on the solid medium, which had the advantage of speed and convenience.

Strains of Staph. aureus *tested*

Tests for egg yolk reaction were made on strains of coagulase-producing staphylococci isolated from burns of in-patients during periods in 1958 (662 strains), in 1960 (457 strains) and in 1962 (329 strains); one strain was selected from each patient at each dressing. We also examined 97 strains from the noses of patients in the burns ward in 1959–60, 202 strains from the noses of patients attending the Casualty Department in 1958, and 134 strains from miscellaneous infections other than burns in 1958.

Other tests on staphylococci

The isolation and examination of these organisms was carried out as described elsewhere (Lowbury, 1960). Staphylococci were tested for phage type by the method described by Blair & Williams (1961) and for sensitivity to antibiotics by a ditch plate method with 10 units of penicillin, 50 μg. of tetracycline and 10 μg. of erythromycin per ml. nutrient agar in the ditches. Mercuric chloride sensitivity was tested in the manner described by Moore (1960).

RESULTS

Table 1 shows the frequency of isolation of egg yolk positive and egg yolk negative strains of *Staph. aureus* from burns in 1958, 1960 and 1962, and from other sources, mostly in 1958; a small number of strains gave weak or doubtful positive reactions, shown separately in this table. In 1958 and again in 1960 a large majority of the strains from burns were EY−, in contrast with the predominantly EY+ strains from miscellaneous infections and from the noses of the general public (represented by patients attending the Casualty Department); the staphylococci from the noses of patients in the burns wards during 1959 and 1960 showed an intermediate proportion of EY− strains, reflecting a replacement of the normal predominantly EY+ staphylococci by strains from burns in a proportion of the patients.

From these data it seemed likely that burns offer some selective advantage to EY− staphylococci. The strains isolated from burns in 1962, however, showed a

different pattern with a preponderance of EY+ strains (though EY− strains were still more common in burns than they were found to be in other environments during 1958).

Phage type and egg yolk reaction

A representative selection of the strains of staphylococci (one strain per patient at each sampling) was phage-typed; the results are presented in Table 2. This shows a preponderance of group III strains in burns during 1958 and 1962, but a slightly greater proportion of group I strains in 1960; the typable staphylococci from other sources were predominantly group I. Staphylococci of group I were predominantly EY+, except those isolated from burns in 1960, 78 % of which were EY−. Staphylococci of group II were rarely found in burns and were all EY+. Staphylococci of group III were usually EY− when isolated from burns, but less often EY− in 1962 than in 1958 and in 1960; the strains of group III from other sources were only slightly more often EY− than strains of group I.

Table 1. *The egg yolk reaction of* Staphylococcus aureus *from burns and from other sources*

Sources of *Staph. aureus*	Numbers of strains tested	Numbers of strains			% strains EY−
		EY+	?EY+	EY−	
Burns in ward, 1958	662	130	2	530	80
Burns in ward, 1960	457	77	11	369	81
Burns in ward, 1962	329	198	11	120	36
Nose and throat of patients in burns ward, 1959–60	97	39	1	57	59
Nose of patients attending Casualty Department, 1958	202	157	1	44	21
Miscellaneous infections in hospital other than burns, 1958	134	106	0	28	21

Table 3 shows the egg yolk reaction of the commoner phage types and patterns (those represented five times or more) of *Staph. aureus* included in Table 1. A striking feature is the presence in burns of 176 strains of type 52; 139 of these were EY−, all of them isolated in 1960 during which year 141 strains of type 52 staphylococci were isolated. This preponderance of one strain explains the high proportion of EY− staphylococci in phage group I during the year 1960 (Table 2); the strains of type 52 in Table 3 which were EY+ were all isolated in 1958 or in 1962. Most of the strains of group I during 1962 were of phage type 80 (or related patterns), and these were EY+. Strains of group II were hardly ever found in burns. In group III there was a preponderance of phage patterns which were predominantly EY−, but also some patterns (e.g. type 53) which were usually EY+.

Antibiotic sensitivity and egg yolk reaction

Table 4 shows the egg yolk reaction and sensitivity or resistance to penicillin, tetracycline and erythromycin of 240 strains of *Staph. aureus* isolated from burns of in-patients in 1958. EY− reactions were more commonly found in strains

Table 2. *Egg yolk reaction and phage group*

Sources of *Staph. aureus*	Strains of *Staph. aureus* in: Phage group I Number	Phage group I % EY⁻	Phage group II Number	Phage group II % EY⁻	Phage group III Number	Phage group III % EY⁻	Other types Number	Other types % EY⁻	Not typable Number	Not typable % EY⁻	Total strains
Noses of patients attending Casualty Department	60	19	36	6	12	27	8	27	84	33	200
Miscellaneous infections other than burns	43	17	16	0	14	22	5	0	22	13	100
Burns in ward, 1958	21	0	0	0	99	97	5	40	13	54	138
Burns in ward, 1960	192	78	3	0	170	90	13	61	79	73	457
Burns in ward, 1962	119	1	3	0	165	66	23	13	19	8	329

resistant to each of the antibiotics; the proportion of antibiotic-sensitive strains was greater among EY+ than among EY− staphylococci, and the majority of EY+ strains were sensitive to tetracycline and to erythromycin.

In Table 3 one phage type of group I (type 52) was predominantly EY−, while other related patterns in the group (52/80, 52/52A/80 and 80) were usually EY+.

Table 3. *Egg yolk reaction of staphylococci from burns and other sources showing the common phage patterns*

Phage pattern	Phage group	Strains from burns		Strains from other sources	
		EY+	EY−	EY+	EY−
29	I	0	0	14	1
29/52	I	0	0	5	1
52	I	35 (2)*	139	24	3
52/52A	I	3	0	4	0
52/80	I	19	1	0	0
52/52A/80	I	31	1	19	2
52A	I	2	6	6	5
52A/79	I	1	0	20	0
52A/80	I	4 (1)	0	4	0
79	I	3	0	19	1
80	I	48 (2)	0	11	0
3B/55/71	II	1	0	4	0
55	II	1	0	8	0
55/71	II	1	0	14	2
71	II	0	0	5	0
6/7/47/53/54/75/75B/77	III	27	6	10	4
53	III	24 (1)	1	2	4
53/75/75B	III	1	4	0	0
53/75/75B/77	III	0	18	0	0
53/75/77	III	0	5	0	0
53/75B/77	III	2 (1)	42	0	0
75/75B	III	1	12	0	2
75/75B/77	III	0 (1)	90	1	1
75B	III	0 (1)	18	3	3
75B/77	III	4 (3)	98	2	0
77	III	3 (1)	60	0	3

* Doubtful positive reactions in brackets.

Similarly, in group III the staphylococci of types 53 and 6/7/47/53/54/75/75B/77 were usually EY+, while other types (e.g. 75/75B/77, 75B/77, 77) were predominantly EY−. Table 5 shows the antibiotic sensitivity patterns of the staphylococci with these phage patterns; both in group I and in group III (especially in the latter) the EY− strains were more commonly resistant to penicillin, tetracycline and erythromycin than the EY+ strains. Since antibiotic resistant strains were more common in burns than in the other environments sampled (Table 6), it seems likely that the frequent selection of EY− strains of group III in burns treated in hospital is due to the selective advantage of antibiotic resistant bacteria in this environment; EY+ strains of type 80, which often show multiple resistance, seem to have a similar advantage.

Egg yolk reaction and mercuric chloride sensitivity

Egg yolk reaction and mercuric chloride sensitivity were tested on 329 strains of *Staph. aureus* from burns of in-patients in 1962.

Table 7 shows the results of these tests. The majority of the strains (255) were resistant to mercuric chloride; the proportion of mercuric chloride resistant ('epidemic') strains was higher among the EY$^-$ staphylococci (111/120) than among those which gave an EY$^+$ reaction (144/209). Out of 26 strains of type 53, only one was EY$^-$, and this was the only strain of type 53 which was mercuric chloride resistant.

Table 4. *Egg yolk reaction and antibiotic resistance*

Antibiotic	Sensitivity test results	240 strains of *Staph. aureus* from burns (1958) EY$^-$	EY$^+$
Penicillin	Resistant	170	60
	Sensitive	1	9
Tetracycline	Resistant	166	21
	Sensitive	5	48
Erythromycin	Resistant	163	24
	Sensitive	8	45

Egg yolk reaction and pigmentation

A large majority of the EY$^+$ strains of staphylococci produced a rich golden-yellow pigment, while EY$^-$ strains were almost always poorly pigmented.

To determine whether the egg yolk reaction and pigmentation were inter-dependent properties, we grew ten pigmented EY$^+$ strains in nutrient broth containing 0·5% (w/v) lithium chloride, a method by which white variants of pigmented *Staph. aureus* have been obtained (Barber, 1955). After repeated daily subcultures, white variants were obtained from a strain of phage type 80 (PS 80). Ten white colonies were picked and found to retain their sensitivity to phage 80 and to give the positive egg yolk reaction shown by the parent strain.

DISCUSSION

Like Gillespie & Alder (1952) and Parker (1958) we found that egg yolk negative strains of *Staph. aureus* were more commonly resistant to antibiotics than egg yolk positive strains, and were more commonly members of phage group III than of phage groups I and II. Staphylococci which colonize burns in hospital have been found predominantly resistant to penicillin, tetracycline and erythromycin, and the selection of these strains would involve the coincidental selection of group III strains and those giving a negative egg yolk reaction. In phage group III the strains which were predominantly egg yolk negative were also the ones showing the highest incidence of multiple resistance and the ones which appeared most frequently in burns. Group I strains which were EY$^+$ (e.g. phage type 80) showed

Table 5. *Antibiotic sensitivity of predominantly EY+ and EY− Staphylococcus aureus within the same phage group*

Phage group	Phage types	Egg yolk reaction	Strains examined	*Staph. aureus* resistant to: Penicillin Number	%	Tetracycline Number	%	Erythromycin Number	%	All these antibiotics Number	%
I	80; 52/80; 52/52A/80; 52A/80	Predominantly EY+	71	68	96	58	82	55	77	48	68
	52	Predominantly EY−	178	176	99	168	94	162	91	157	88
III	6/7/47/53/54/75/75B/77, and closely related patterns; 47/77; 53; 6	Predominantly EY+	39	30	77	16	41	19	49	10	26
	53/75/75B/77; 53/75/77; 53/75B/77; 75/75B/77; 75/75B; 75B	Predominantly EY−	245	240	98	185	76	232	95	175	71

Table 6. *Antibiotic resistance of staphylococci from different environments*

Source of *Staph. aureus*	Strains of *Staph. aureus* resistant to: Penicillin Number	%	Tetracycline Number	%	Erythromycin Number	%
Burns, 1958	230	96	187	78	187	78
Casualty patients, 1958	18	9	2	1	0	0
Infections other than burns	43	43	30	30	2	2

multiple resistance to antibiotics as often as EY⁻ strains and appeared to colonize burns as successfully as any of the EY⁻ strains of group III; in 1962 these strains were slightly more common than the EY⁻ strains of group III and became the predominant staphylococci of the Burns Unit. An example of a change in which one phage type (probably a single strain) became predominant was the emergence of type 52 EY⁻ strains in 1960. These staphylococci were indistinguishable by phage typing from the smaller number of type 52 strains isolated from burns in 1958, but egg yolk testing showed the latter to be all EY⁺. In differentiating between strains of the same phage type or between strains which are untypable, the egg yolk reaction appears to have some value as a test in epidemiological studies of staphylococcal infection.

Table 7. *Egg yolk reaction and mercuric chloride resistance*

	Numbers of strains of *Staph. aureus*			
Egg yolk reaction	Mercury resistant	Mercury sensitive	Doubtful reaction	Total
Positive	134	54	10	198
Doubtful positive	10	1	0	11
Negative	111	5	4	120
Total	255	60	14	329

The relevance of the egg yolk reaction to virulence and epidemicity is uncertain. Gillespie & Alder (1952) found that EY⁺ strains were more commonly associated with invasive infection, and EY⁻ strains were more commonly associated with superficial lesions such as impetigo and open wounds. Jessen, Faber, Rosendal & Eriksen (1959) found that EY⁻ strains were the commonest of the antibiotic-resistant staphylococci causing bacteraemia. These authors agree in classifying EY⁻ strains as pathogens, while apparently differing in their assessment of the invasiveness of such strains; a different view is implicit in the use by some authors of the egg yolk reaction as a diagnostic criterion for pathogenic staphylococci (e.g. Baird-Parker, 1962). Staphylococci-colonizing burns usually cause no obvious pathological effects, but on occasions they may invade the blood stream causing septicaemia or pyaemia; both EY⁻ and EY⁺ strains have been isolated from blood cultures in such cases. The majority of strains isolated from burns were mercury-resistant, and therefore 'presumptive epidemic strains'; of the mercury-sensitive strains a larger proportion gave EY⁺ than EY⁻ reaction.

These studies suggest that neither the egg yolk reaction (i.e. the production of a specific lipase) nor the phage group in themselves confer a selective advantage on particular staphylococci in burned tissue. Certain staphylococci, especially those of group III (Barber & Whitehead, 1949), appear to produce more mutations than others, and mutants which are better adapted because of antibiotic resistance or other features can be expected to flourish and eventually displace the less well adapted strains. A surprisingly large proportion of the staphylococci isolated from burns at certain times appeared to be 'subcultures' of the same strain; the pre-

ponderance of a single strain accounts for the fact that most staphylococci of group I were EY⁻ in 1960 and that EY⁺ staphylococci became the commonest burn staphylococci in 1962.

SUMMARY

Staph. aureus from burns of in-patients were tested for egg yolk reaction during three periods; in 1958 and in 1960 approximately 80% of the strains gave a negative reaction (EY⁻), but in 1962 only 36% of the strains were egg yolk negative.

Staphylococci of phage group III were more commonly EY⁻ than those of other groups isolated from burns. Within each of groups I and III, however, there were patterns predominantly EY⁻ and others predominantly egg yolk positive (EY⁺); in group I the majority of strains isolated in 1960 were of phage type 52 and EY⁻, while those isolated in 1962 were predominantly of phage type 80 or related patterns which were always EY⁺.

Most of the staphylococci in burns were resistant to penicillin, tetracycline and erythromycin; within groups I and III, the staphylococci which were EY⁻ were also more commonly resistant than EY⁺ strains to these three antibiotics.

Most of the staphylococci from burns were mercuric chloride resistant (presumptive epidemic strains); of the mercuric chloride sensitive staphylococci, the proportion of EY⁺ strains was greater than that of EY⁻ strains.

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