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Agar Medium for the Selective Enumeration of Coagulase-positive *Staphylococcus* from the Rat Alimentary Tract

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A selective agar medium (medium J_1) is proposed for the quantitative enumeration of egg yolk-positive (EYP) and egg yolk-negative (EYN) *Staphylococcus pyogenes* from the digestive tract and feces of the rat. This medium, buffered at *p*H 5.0, is composed of acid casein hydrolysate and yeast extract with 7.5% sodium chloride, 1.6% sodium pyruvate, 0.0008% 2,3,5-triphenyltetrazolium chloride (TTC), and 6% egg yolk emulsion. Inoculation is by the pour-plate method and incubation is at 38 C in a water-jacketed incubator for 36 hr. Colonies of *S. pyogenes* reduce TTC; EYP strains are surrounded by a halo of opacity; and EYN strains may be surrounded by a red halo, but no opacity. Small, white colonies of *S. epidermidis* may develop, but *Micrococcus*, and all other groups of *Staphylococcus* recognized in the rat intestinal flora, are inhibited. Other bacterial genera, notably *Bacillus*, *Corynebacterium*, *Proteus*, and *Streptococcus*, are also inhibited.

The literature reveals that much painstaking work has been done in the past in the attempt to isolate *Staphylococcus pyogenes*. There are at least 62 publications wherein 48 agar media for the isolation of *S. pyogenes* are described. These are media devised for use with material examined for medical, veterinary, public health, and food-control purposes. Complete correlation between the ability to coagulate plasma and any other biochemical test used for the recognition of *S. pyogenes* may not be achieved (4, 10). Thus, the only sure indication of *S. pyogenes* in an initial isolation medium is the demonstration of plasma coagulation. Unfortunately, solid media containing plasma and fibrinogen are insufficient for the recognition of coagulase-positive colonies, since many false-negative results are obtained under these conditions (21).

Among the many media that have been evolved, no single medium exists that allows the unique isolation of *S. pyogenes*. In spite of differential aids, particular difficulty may be experienced when material containing other members of the *Micrococcaceae* is examined. The very fact that such a large number of selective media exists indicates, as stated by Smuckler and Appleman (33), that "the perfect medium has not been developed."

The object of the present work was to attempt to create such a medium for the selective enumeration of *S. pyogenes* and for the isolation of this organism from the alimentary tract and feces of the albino rat, in the presence of overwhelming numbers of aerobes and other facultative anaerobes belonging to the *Micrococcaceae* and other bacterial families (26).

MATERIALS AND METHODS

Origin, isolation, and culture of strains employed during trials. Samples of the stomach and intestine (including the wall), and of feces, of "holoxenic" (conventional; 28) albino rats prepared during the analysis of the gastrointestinal microflora (25, 26) were employed. Decimal dilutions of samples were prepared in "diluent II" (25), which contains [%(w/v) in distilled water]: vitamin-free casein hydrolysate (acid), salt-free (Nutritional Biochemicals Corp., Cleveland, Ohio), 0.2; yeast extract (Difco), 0.1; and NaCl, 0.5 (*p*H 7.0). Appropriate dilutions were plated in medium "H_1" (25). As described previously, the primary medium, "H" (25), is composed of "diluent II" with the addition of (%, w/v): agar (Difco), 2.0; and phenol red (Eastman-Kodak, Rochester, N.Y.), 0.0012. The technique (24) involves a preliminary contact at 37 C for 5 min between the bacteria in 1 ml of each dilution and the inhibitor and thermolabile substances in an "intermediate tube." The composition [% (w/v) in distilled water] and quantities of these Seitz-filtered substances in the "intermediate tubes" are as follows: 30% sodium acetate (E. Merck AG., Darmstadt, W. Germany) with 0.03% potassium tellurite (British Drug Houses Ltd., Poole, England),

1 ml; 30% urea (Prolabo, Rhône-Poulenc, Paris, France), 0.5 ml; and 10% egg lecithin (Nutritional Biochemicals Corp.), 0.3 ml. The contents of the "intermediate tubes" after "contact" are poured, with 15 ml of molten, cooled primary medium "H", into petri plates. The resultant secondary medium has been designated medium "H_1." Colonies representative of *Staphylococcus*, *Micrococcus*, *Streptococcus*, *Bacillus*, *Corynebacterium*, *Escherichia*, *Lactobacillus*, and *Proteus* were isolated (26). After purification on blood agar plates [Blood Agar Base (Difco) containing 52% sheep blood], isolates of *Staphylococcus* and *Micrococcus* were maintained on the "tryptone medium" for indole production (9), and 6-hr cultures in this medium were used for inoculating trial media. Additional strains of *Staphylococcus* that were isolated in medium "H_1" came from "heteroxenic" [specific pathogen-free (SPF)] (28) rats of the first colony described (27), and two strains of *S. pyogenes* came from "heteroxenic" mice reared with this colony. Some samples of the alimentary tract of "gnotoxenic" (gnotobiotic; 28) rats were included in the experiments. Strains of *S. pyogenes* [egg yolk-positive (EYP) and egg yolk-negative (EYN)] and of *S. epidermidis* isolated from man were kindly supplied by V. G. Alder and W. A. Gillespie.

Identification of Staphylococcus and Micrococcus. Distinction of strains of catalase-positive cocci into the genera *Staphylococcus* and *Micrococcus* was made on the basis of anaerobic production of acid from glucose, as recommended by the Subcommittee on Taxonomy of Staphylococci and Micrococci (34). For this, 15 ml of GA medium (9) was inoculated when molten with 0.1 ml of strains cultured for 18 hr at 37 C in the "tryptone medium"; the inoculated medium was poured into 8 × 400 mm tubes (25) and immediately cooled. Incubation was for 1 week at 38 C, and tubes were examined daily for growth and indicator change. The species *S. pyogenes* was recognized on the basis of its ability to coagulate rabbit plasma. The plasma and "bouillon" for the test were obtained from the Institut Pasteur, Paris, France. Preliminary work (*to be reported*) on physiological and biochemical characters of the isolates included the following tests, for which all media were inoculated with 0.1 ml of 18-hr cultures incubated at 37 C in the tryptone medium.

Growth in a liquid glucose-containing medium. Quantities of 5 ml of a medium containing [%(w/v) in deionized water] 1.0% tryptone (Difco), 0.5% yeast extract, 0.5% NaCl, and 0.5% glucose (11) were dispensed in tubes (16 × 160 mm) and sterilized (118 C for 20 min). The contents of two tubes for each strain were examined for *p*H (EIL *p*H meter) and turbidity (EIL nephelometer) after 1, 2, 3, 4, 5, and 7 days of incubation at 37 C.

"TTC test" at pH 5.0 and pH 6.0. A medium (medium YP) containing [% (w/v) in distilled water] 0.1% yeast extract (Difco), 2.78% NaH_2PO_4, and 1.5% agar (Difco), at *p*H 5.0 or 6.0 before a primary sterilization for 10 min at 120 C, was filtered, dispensed in 2.5-ml quantities, and sterilized at 115 C momentarily. Medium YP was inoculated in the manner described above for medium "H_1," poured into Veillon tubes (8 × 18 mm), and plunged immediately into cold water. The "intermediate tubes" contained 0.1 ml of a Seitz-filtered solution, in distilled water, of 0.15% 2,3,5-triphenyltetrazoluim chloride (TTC; E. Merck AG.) and 15% sodium pyruvate (E. Merck AG.). The Veillon tubes were examined for growth and reduction of TTC after 36 hr at 37 C.

Egg yolk opacity in liquid media. Egg yolk opacity was tested with one EYP strain of *S. pyogenes* by use of the medium described by Gillespie and Alder (12) and a "pyruvate medium" composed of "diluent II" containing 2% sodium pyruvate (E. Merck AG.) sterilized at 115 C for 20 min. After sterilization, 6% egg yolk emulsion was added. Egg yolk emulsion was prepared by a modification of Billing and Luckhurst's method (6) in which a sterile "Chardin no. 11 bis" filter paper was used. Commercial egg yolk emulsion (Oxoid) was also used. Incubation was at 37 C for 14 hr, and 0.1 ml of *Staphylococcus* antitoxin (1,000 units/ml), prepared by Burroughs Wellcome & Co. (London, England) was added to tubes containing 5 ml of the two media before inoculation.

Egg yolk opacity reaction in agar media. Two media were employed: one consisted of "diluent II" with 2% agar (Difco), and the other was medium J_1 (*see below*). The concentrations of added substances and the method of inoculation were identical to those given for medium J_1. In some cases, 0.6 ml of *Staphylococcus* antitoxin was added to the "intermediate tube" (Table 3).

Trial media. Three trial media were used: medium 1 contained "diluent II" with 2% agar (Difco); medium 2 was medium 1 with a final concentration of 7.5% NaCl; medium 3 was the same as medium 2 but was adjusted to *p*H 5.0 and contained 2.78% NaH_2PO_4 as a buffer. All three media contained 1.7% sodium pyruvate, added by the technique described for medium "H_1" (the "intermediate tubes" contained 1 ml of 30% sodium pyruvate at *p*H 7.0). For anaerobic culture, the inoculated media were transferred to 8 × 400 mm tubes. All trial media were incubated at 38 C for 36 hr.

Selective medium J_1 proposed for the isolation of S. pyogenes. Medium J_1 is prepared in two parts. Primary medium J contains [% (w/v) in distilled water]: vitamin-free casein hydrolysate (acid) salt-free (Nutritional Biochemicals Corp.), 0.2; yeast extract (Difco), 0.1; NaH_2PO_4, 2.78; NaCl, 7.5; and agar (Difco), 2.0. After dissolution, the medium is adjusted to *p*H 5.1, autoclaved at 120 C for 10 min, and filtered through a Durieux no. 127 paper with Hyflo Super-Cel (Johns-Manville, New York, N.Y.) as a filter aid. After the addition of 0.0012% phenol red (Eastman Kodak, Rochester, N.Y.), 15-ml quantities of the medium are dispensed in tubes (18 × 180 mm) that are then autoclaved momentarily at 115 C. The final *p*H of the medium is 5.0. The tubed medium may be stored at 4 C for no longer than 1 month. For secondary medium J_1, "intermediate tubes" are prepared, immediately prior to use, containing 0.1 ml of 0.15% TTC, 1 ml of 30% sodium pyruvate at *p*H 7.0, and 1 ml of egg yolk emulsion. The technique of inoculation to give the selective medium J_1 is identical to that described for medium H_1, and incubation is for 36 hr at 38 C in a water-jacketed incubator.

RESULTS

Since strains of coagulase-negative cocci are among the most difficult organisms to eliminate from selective media for *S. pyogenes*, an initial study was undertaken of the physiological and biochemical characters of the various groups of *Micrococcaceae* isolated by means of medium H_1 from the rat alimentary tract. Three groups of *Staphylococcus* (I to III), one "intermediate group" (IVa and b), and three groups of *Micrococcus* (V to VII) were recognized during this study. Results obtained in the first two of the following tests provided indispensable information for the selective isolation of *S. pyogenes* (group I) from the ecological system existing in the rat alimentary tract.

Final pH of strains of Micrococcaceae in a liquid glucose-containing medium. The final *p*H was 4.3 to 4.4 for strains of *S. pyogenes* group II and certain group IV strains, and was between 5.0 and 5.4 for all other strains.

Inhibitory effect of pH and TTC on growth of strains of Micrococcaceae. Only *S. pyogenes* group II and certain strains of group IV were able to grow at *p*H 5.0 in the TTC test (Table 1). Strains of *S. pyogenes* gave a red band of growth 0.1 to 0.2 cm below the surface. Strains of group II and certain strains of the "intermediate group" (group IVa) grew in a red band at approximately 0.5 cm from the surface. Other coagulase-negative strains of the genus *Staphylococcus* (groups III and IVb) were inhibited. Strains of the genus *Micrococcus* tested (groups V, VI, and VII) were all unable to grow at *p*H 5.0, but grew in a red band 0.2 cm from the surface at *p*H 6.0.

Influence of pH, sodium chloride, TTC, and egg yolk on the selective isolation of S. pyogenes. Comparative studies in plated media on pure cultures of strains of *Micrococcaceae* belonging to the various groups recognized, and of a strain of *Streptococcus faecalis* are given in Table 2. Quantitative growth in medium J_1 was obtained for all six strains of *S. pyogenes* after 36 hr, and for strains of groups II and IVa after 48 hr. Strains of all other groups were inhibited after 36 hr, although after 48 hr strains of group V gave small white colonies.

Egg yolk opacity reaction of S. pyogenes in the presence of sodium pyruvate. An EYP strain of *S. pyogenes* isolated from a "holoxenic" rat tested by the method of Gillespie and Alder (12) gave a heavy flocculent precipitate in the pyruvate liquid medium, whereas no reaction occurred in this medium with egg yolk alone, even on incubation for a further 6 days. Reactions of this EYP strain of *S. pyogenes* in various egg yolk media are given in Table 3. The clear halo seen on the opaque media was not visible in media containing 7.5% NaCl, since the background is transparent. A heavy precipitate was produced by surface and deep colonies at *p*H 5.0, particularly in the presence of 7.5% NaCl. Opacity production was inhibited in solid and liquid media at both *p*H 5.0 and 7.0 by *Staphylococcus* antitoxin. All reactions on egg yolk were prevented in agar media by this antitoxin.

Results obtained with the selective medium proposed. After 36 hr at 38 C, EYP strains of *S. pyogenes* appeared as red 0.5-mm lenticular or double lenticular colonies surrounded by a halo of opacity (Fig. 1). EYN strains of *S. pyogenes* (received from V. G. Alder and W. A. Gillespie) gave red colonies that were slightly smaller (0.3 mm), surrounded by a small red halo but no opacity. Although retarded, group II strains and strains of *S. epidermidis* supplied by Drs. Alder and Gillespie are capable of growing on the me-

TABLE 1. *"TTC test" results for strains of all groups of Micrococcaceae isolated from the rat alimentary tract*

Experimental group	No. of strains tested	Coagulase	TTC test			
			*p*H 5.0		*p*H 6.0	
			Growth	Distance from surface (cm)	Growth	Distance from surface (cm)
Staphylococcus						
I	90	+	+	0.2	+	0.2
II	14	−	+	0.5	+	0.5
III	50	−	−		−	
Intermediate group						
IVa	10	−	+	0.2–0.5	+	0.2–0.5
IVb	20	−	−		±	0.2–0.5
Micrococcus						
V, VI, VII	212	−	−		+	0.2

TABLE 2. *Enumeration in trial agar media and medium J_1 of representative strains of all groups of Micrococcaceae and of Streptococcus faecalis isolated from the rat alimentary tract: effect of NaCl, pH, TTC, and egg yolk*[a]

Genus	Experimental group	No. of strains	Trial media					Medium J_1	
			1		2		3	36 hr	48 hr
			Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic		
Staphylococcus	I	6	+	+	+4 −2[b]	−	−	+ Red, halo of opacity	+
	II	2	+	+	+	+	−	(+) White	+ Red
	III	2	+	+	+	−	−	−	−
Intermediate group	IV (a)	1	+	+	+	−	−	(+) White	+ Red
	(b)	2	−	−	−	−	−	−	−
Micrococcus	V	4	+	−	+	−	−	−	+ White
	VI	4	+	−	+	−	−	−	−
	VII	4	+	−	+	−	−	−	−
Streptococcus faecalis		1	(+)	+	−		−	−	−

[a] Symbols: +, quantitative growth; (+), growth slightly inhibited; −, no growth.
[b] Strains isolated from "heteroxenic" (SPF) mice.

TABLE 3. *Reaction of Staphylococcus pyogenes in agar-egg yolk media: effect of pH, NaCl, sodium pyruvate, and Staphylococcus antitoxin*

Characteristic	Reaction in agar-egg yolk media											
	Without sodium pyruvate, pH 7.0		With sodium pyruvate									
			pH 7.0		pH 7.0 with *Staphylococcus* antitoxin		pH 5.0		pH 5.0 with NaCl		pH 5.0 with NaCl and *Staphylococcus* antitoxin	
	Surface	Deep	Surface	Deep	Surface	Deep	Surface	Deep	Surface	Deep	Surface	Deep
Opacity	− (slight)[a]	+ (slight)	−[a]	+ (narrow)	−	−	+ (narrow)	+	+	+	−	−
Clear halo	+	+	+	+	−	−	+	+	−	−	−	−
"Pearly" surface layer[b]	+	−[a]	−[a]	−[a]	−	−	−[a]	−[a]	−[a]	−[a]	−	−

[a] Positive reaction at 2 to 3 days of incubation.
[b] Willis and Turner (35).

dium. They appear as small (0.3 mm) white colonies at 36 hr and deepen to red at 48 hr. Table 2 shows that strains belonging to all other groups of the *Micrococcaceae* isolated from the rat alimentary tract were inhibited on this medium at 36 hr. Pure strains of *Bacillus*, *Corynebacterium*, *Proteus*, and *Streptococcus faecalis* were also inhibited. However, 100 strains of *S. pyogenes* isolated from the rat grew quantitatively in medium J_1, and *S. pyogenes* is the only organism which has grown and been enumerated in this medium during examination of 260 samples from 287 rats during the period from 1963 to 1966. A comparison between medium J_1 and three previously described selective media containing egg yolk was made with three samples of the rat alimentary tract (Table 4). *Corynebacterium*, *Streptococcus faecalis*, *Lactobacillus*, *Escherichia*, and other members of the *Micrococcaceae* were present. It may be seen that EYP strains of *S. pyogenes*

could generally be enumerated on all the media tested. However, of all the bacteria present, only *S. pyogenes* grew on medium J_1. This medium is, therefore, superior to the others for the quantitative isolation of strains of *S. pyogenes* in pure culture from rat alimentary tract contents. Experimental results in vitro suggest that EYN strains of *S. pyogenes* may also be isolated quantitatively by use of this medium.

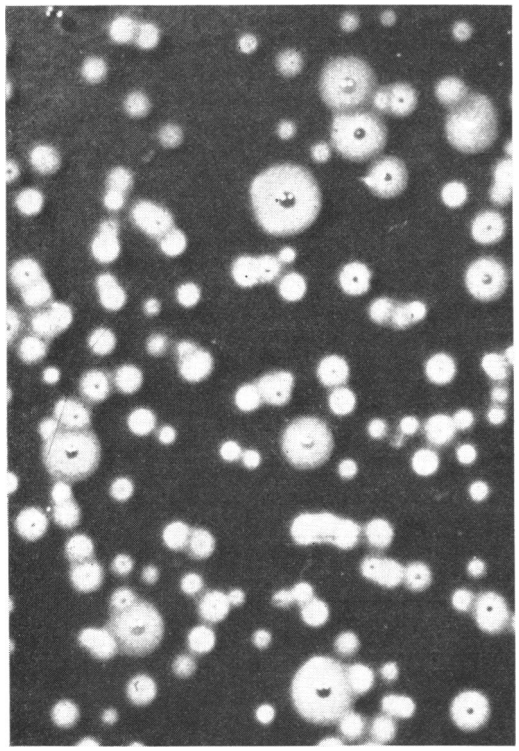

FIG. 1. *Colonies of EYP coagulase-positive Staphylococcus in medium J_1 [10^{-2} dilution of the intestine from a "holoxenic" (conventional) rat].*

Rhizoid colonies of moulds and small lenticular colonies of yeasts were detected on medium J_1 in samples from the feces of a "heteroxenic" rat and mouse, respectively. However, no moulds or yeasts developed on medium J_1 from feces of "holoxenic" rats, although they were known to be present in certain samples.

DISCUSSION

The following factors govern the selective action of medium J_1 here described: a high concentration of sodium chloride, now a classical method for the selective inhibition of gram-negative and some gram-positive facultative aerobes; a limited nutrient supply, chosen for good growth of *S. pyogenes* and reduction of growth of other bacteria, combined with TTC; and a low *p*H. The use of TTC and the low *p*H are necessary for the further inhibition of other gram-positive facultative aerobes, particularly cocci, present in the intestinal microflora and feces of the rat.

Sodium pyruvate was used as the main carbon source in the medium, since, of all organic acid salts tested during the present work, pyruvate supported the best growth of *S. pyogenes* and suppressed aerobic growth of a test strain of *Streptococcus faecalis* (Table 2). Baird-Parker (3) included sodium pyruvate in the selective medium he proposed, since he observed that, in comparison with *S. pyogenes*, "micrococci" metabolized this compound slowly or not at all. However, pyruvate, although necessary, is not sufficient for growth of *S. pyogenes* in medium

TABLE 4. *Comparison of results obtained on several egg yolk media with samples of the alimentary tract (stomach + intestine) of "holoxenic" (conventional) rats*

Bacterial group	Age of rat sampled (days)	Enumeration[a] on egg yolk media[b]					Enumeration[a] on media selective[c] for		
		1	2	3	4	5	*Escherichia*	*Streptococcus*	*Lactobacillus*
Staphylococcus pyogenes	15	5.2	5.3	5.3	5.2	5.0			
Other genera		0	8.0	7.0	7.0	7.0	6.5	7.5	8.3
S. pyogenes	10	4.9	4.9		4.9	4.3			
Other genera		0	5.8		5.0	6.2	7.4	5.5	7.6
S. pyogenes	5	2.0	2.3	<1.0	2.0	<2.0			
Other genera		0	5.0	3.9	2.3	5.0	<2.0	5.6	9.0

[a] Log_{10} number of bacteria per gram of fresh sample.

[b] 1, Medium J_1; 2, Baird-Parker ETGPA medium (3); 3, SM "110 + egg yolk; 4, Carantonis and Spink medium (7); 5, Medium "H_1" (25).

[c] Raibaud, Dickinson, Sacquet, Charlier and Mocquot (25)

J_1, since either egg yolk (Table 2) or egg lecithin (0.1%) is also indispensable.

Among the chemical inhibitors that have been used in media containing a high concentration of sodium chloride, TTC was previously used with partial success by Kennedy and Barbaro (19), for the differentiation of *S. pyogenes* from coagulase-negative "micrococci," by appreciation of colony size. However, over 25% of the coagulase-negative strains of "micrococci" from human sources gave colonies identical in size to those of *S. pyogenes* after 24 hr in their medium. Since colonies of *S. pyogenes* reduce TTC after 36 hr in medium J_1, they may be distinguished from those of *S. epidermidis*, which remain white under these conditions. In addition, other members of the *Micrococcaceae* capable of reducing TTC in the "TTC test" at *p*H 6.0, are unable to grow on medium J_1.

Among the physical agents used for the isolation of staphylococci, the selective effect of *p*H 8.6 or 8.8 to 9.0 has been observed previously (5, 8). However, up to the present, the differential effect of an agar medium adjusted to *p*H 5.0 on the growth of species of the *Micrococcaceae* has not been observed.

Egg yolk, the differential agent employed in medium J_1, is included for two reasons: (i) to supply sufficient nutrients, and (ii) to distinguish between EYP and EYN strains of coagulase-positive staphylococci.

The production of opacity by certain coagulase-positive strains of *Staphylococcus*, was originally observed by Gillespie and Alder (12). Equivalent results for the egg yolk opacity reaction have not always been obtained in agar media containing egg yolk (31) with strains that were positive in the test of Gillespie and Alder (12). The opacity reaction in a solid medium becomes more easily visible when the egg yolk in the medium is clarified either by adjusting the *p*H value above 8.0 (14) or by adding 1% or more sodium chloride (15). There is no information in the literature on the influence of high concentrations of sodium chloride on the egg yolk opacity reaction; however, the results obtained during this work show that 7.5% NaCl has no adverse influence on the opacity reaction produced by colonies of EYP *S. pyogenes* in solid egg yolk media (Table 3). In fact, the conditions in medium J_1 appear to enhance the egg yolk opacity reaction. This may be due to the choice of *p*H, since this is near to one of the optima described for the egg yolk opacity reaction (32), it may be due to the presence of sodium pyruvate, or it may be due to a greater diffusion of the enzyme into the surrounding soft medium [large halos were also obtained on the high salt medium of Carantonis and Spink (7)]. It is interesting to note from the experimental results that only the opacity reaction is produced by both surface and deep colonies in the medium at the selected *p*H value of 5.0 by a representative EYP strain of *S. pyogenes*, and that the clear halo and "pearly" surface layer (35) are inhibited (Table 3). The opacity reaction observed in the presence of sodium pyruvate may be considered identical to that described by Gillespie and Alder (12), since *Staphylococcus* antitoxin has had the same inhibitory action on its production. Formation of a clear halo and a "pearly" surface layer at *p*H 7.0 by colonies of EYP *S. pyogenes* appears to be closely related to the production of opacity, since all three reactions were prevented, at this *p*H, by *Staphylococcus* antitoxin. Results obtained during the present work refute the statement made by Pernice and Macri (23) that, contrary to the observations of Gillespie and Alder in a liquid medium (12), a solid medium at acid *p*H values does not favor the enzymatic activity involved in the egg yolk opacity reaction.

Egg yolk has been incorporated as a differential aid into several selective isolation media where colonies of coagulase-positive staphylococci have been differentiated by the production of a clear halo or opacity. Unfortunately, colonies of coagulase-negative strains of *Micrococcaceae* grow on all these media, and on all except Innes' medium (15) they may give a reaction similar to that of coagulase-positive strains. The latter medium is, however, too inhibitory to *S. pyogenes* (2).

Certain coagulase-positive staphylococci of human (1, 7, 13, 16, 22, 20, 31) and animal (1, 29) origin have been recognized to be EYN, even under optimal conditions for the egg yolk opacity reaction. EYN strains of coagulase-positive staphylococci have been involved in human cases of bacteremia, where mortality was twice as high as for cases caused by EYP strains (17, 18, 30). Even certain EYN coagulase-negative staphylococci have been considered to be pathogenic, for they have proved to be phage-typable (7). Thus, the medium presently described offers an advantage over previous egg yolk selective media in that only *S. pyogenes* develops quantitatively. EYN strains may thus be distinguished immediately. The only other bacteria that may develop are the closely related *S. epidermidis* strains (group II) that grow more slowly and may easily be recognized by the fact that TTC is not reduced after 36 hr of incubation.

During the experimental work, it was noticed that coagulase-positive staphylococci in pure culture, and at isolation from the intestinal tract of "monoxenic" (28) rats, grew quantitatively after surface inoculation of medium J_1 by spread-

ing 0.1 ml of the appropriate decimal dilutions. However, a sample of the intestinal contents of a "holoxenic" animal containing 10^5 viable *S. pyogenes* cells showed no growth after surface inoculation of medium J_1. It must, therefore, be noted that the medium described is designed for pour-plate inoculation, and thus differs from the majority of selective media for the isolation of *S. pyogenes*, and from all such media containing egg yolk.

It must also be pointed out that, if additional nutrients were added to the medium, its selectivity could be lost. The contents of the alimentary tract (diluted to 10^{-1}) of all rats examined, however, did not alter the specificity of the medium. Lipases originating from fragments of tissue or food may sometimes cause opacity of the egg yolk, particularly at the 10^{-1} dilution of samples.

Since this medium allows the detection and quantitative isolation of coagulase-positive *Staphylococcus*, even at the 10^{-1} dilution of alimentary tract contents, it may prove to be indispensable for following the development of strains of this organism during implantation studies in the alimentary tract of the "gnotoxenic" rat under different conditions of rearing. In this way, the interactions and ecological relationships in vivo of the associated microflora may be elucidated.

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LITERATURE CITED

1. ALDER, V. G., W. A. GILLESPIE, AND G. HERDAN. 1953. Production of opacity in egg-yolk broth by staphylococci from various sources. J. Pathol. Bacteriol. **66**:205–210.
2. APPLEMAN, M. D., N. BAIN, AND J. M. SHEWAN. 1964. A study of some organisms of public health significance from fish and fishery products. J. Appl. Bacteriol. **27**:69–77.
3. BAIRD-PARKER, A. C. 1962. An improved diagnostic and selective medium for isolating coagulase positive staphylococci. J. Appl. Bacteriol. **25**: 12–19.
4. BAIRD-PARKER, A. C. 1963. A classification of micrococci and staphylococci based on physiological and biochemical tests. J. Gen. Microbiol. **30**:409–427.
5. BEVERIDGE, W. I. B. 1940. An alkaline culture medium for differentiation of staphylococci and micrococci from cow's milk. Australian J. Exptl. Biol. Med. Sci. **18**:391–392.
6. BILLING, E., AND E. R. LUCKHURST. 1957. A simplified method for the preparation of egg yolk media. J. Appl. Bacteriol. **20**:90.
7. CARANTONIS, L. M., AND M. S. SPINK. 1963. A selective salt egg agar medium for pathogenic staphylococci. J. Pathol. Bacteriol. **86**:217–220.
8. CHAPMAN, G. H., C. W. LIEB, C. BERENS, AND L. G. CURCIO. 1937. The isolation of probable pathogenic staphylococci. J. Bacteriol. **33**:533–543.
9. DICKINSON, A. B., AND G. MOCQUOT. 1961. Studies on the bacterial flora of the alimentary tract of pigs. I. Enterobacteriaceae and other Gram-negative bacteria. J. Appl. Bacteriol. **24**:252–284.
10. ELEK, S. D. 1959. *Staphylococcus pyogenes* and its relation to disease. E. & S. Livingstone Ltd., Edinburgh and London.
11. EVANS, J. B., AND C. F. NIVEN, JR. 1950. A comparative study of known food-poisoning staphylococci and related varieties. J. Bacteriol. **59**: 545–550.
12. GILLESPIE, W. A., AND V. G. ALDER. 1952. Production of opacity in egg-yolk media by coagulase-positive staphylococci. J. Pathol. Bacteriol. **64**:187–199.
13. GRABER, C. D., R. LATTA, J. P. FAIRCHILD, AND E. H. VOGEL. 1958. Production of opalescence by staphylococci in egg yolk medium as an index bacteriophage typability. Am. J. Clin. Pathol. **30**:314–317.
14. HOPTON, J. 1961. A selective medium for the isolation and enumeration of coagulase positive staphylococci from foods. J. Appl. Bacteriol. **24**:121–124.
15. INNES, A. G. 1960. Tellurite-egg agar, a selective and differential medium for the isolation of coagulase positive staphylococci. J. Appl. Bacteriol. **23**:108–113,
16. JESSEN, O., V. FABER, K. ROSENDAL, AND K. R. ERIKSEN. 1959. Some properties of Staphylococcus aureus, possibly related to pathogenicity. I. A study of 446 strains from different types of human infection. Acta Pathol. Microbiol. Scand. **47**:316–326.
17. JESSEN, O., V. FABER, K. ROSENDAL, AND K. R. ERIKSEN. 1959. Some properties of Staphylococcus aureus, possible related to pathogenicity. II. In vitro properties and origin of the infecting strains correlated to mortality in 190 patients with Staphylococcus aureus bacteremia. Acta Pathol. Microbiol. Scand. **47**:327–335.
18. JESSEN, O., K. ROSENDAL, V. FABER, K. HOVE, AND K. R. ERIKSEN. 1963. Some properties of Staphylococcus aureus, possibly related to pathogenicity. Part 3: Bacteriological investigations of Staphylococcus aureus strains from 462 cases of bacteraemia. Acta Pathol. Microbiol. Scand. **58**:85–98.
19. KENNEDY, E. R., AND J. F. BARBARO. 1952. The inhibitory effect of triphenyltetrazolium on some strains of micrococci. J. Bacteriol. **63**:297–298.
20. LOWBURY, E. J. L., AND B. J. COLLINS. 1964. The

egg yolk reaction of *Staphylococcus aureus* isolated from burns. J. Hyg. **62**:229-237.

21. McDIVITT, M. E., AND N. W. JEROME. 1965. Limitations of fibrinogen-polymyxin medium in detecting coagulase-positive staphylococci in raw milk. Appl. Microbiol. **13**:157–159.
22. PARKER, M. T. 1958. Some cultural characteristics of *Staphylococcus aureus* strains from superficial skin infections. J. Hyg. **56**:238–253.
23. PERNICE, A., AND N. MACRI. 1962. Influenza del *p*H sulla attività enzimatica dello stafilococco nei terreni al giallo d'uova. 1) Comportamento sui terreni solidi. Giorn. Batteriol. Virol. Immunol. **55**:227–235.
24. RAIBAUD, P., M. CAULET, J. V. GALPIN, AND G. MOCQUOT. 1961. Studies on the bacterial flora of the alimentray tract of pigs. II. Streptococci: selective enumeration and differentiation of the dominant group. J. Appl. Bacteriol. **24**:285–306.
25. RAIBAUD, P., A. B. DICKINSON, E. SACQUET, H. CHARLIER, AND G. MOCQUOT. 1966. La microflore du tube digestif du rat. I. Techniques d' étude et milieux de culture proposés. Ann. Inst. Pasteur **110**:568–590.
26. RAIBAUD, P., A. B. DICKINSON, E. SACQUET, H. CHARLIER, AND G. MOCQUOT. 1966. La microflore du tube digestif du rat. II. Dénombrement de différents genres microbiens dans l'estomac et l'intestin de rats conventionnels. Variations quantitatives individuelles et en fonction de l' âge. Ann. Inst. Pasteur **110**:861–876.
27. RAIBAUD, P., A. B. DICKINSON, E. SACQUET, H. CHARLIER, AND G. MOCQUOT. 1966. La Microflore du tube digestif du rat. III. Implantation fortuite de différents genres microbiens chez le rat indemne de microbes pathogènes spécifiques (rat "SPF"). Ann. Inst. Pasteur **111**:46–56.
28. RAIBAUD, P., A. B. DICKINSON, E. SACQUET, H. CHARLIER, AND G. MOCQUOT. 1966. La microflore du tube digestif du rat. IV. Implantation contrôlée chez le rat gnotobiotique de différents genres microbiens isolés du rat conventionnel. Ann. Inst. Pasteur **111**:193–210.
29. REID, W. B., AND J. B. WILSON. 1959. A study of the staphylococci associated with the bovine udder. Am. J. Vet. Res. **20**:825–831.
30. ROSENDAL, K. 1962. Correlation of virulence of staphylococci from patients with bacteraemia with various laboratory indices, p. 570–574. *In* N. E. Gibbons [ed.], Recent progress in microbiology. Symp. Intern. Congr. Microbiol., 8th, Montreal. Univ. Toronto Press, Toronto.
31. SHAH, D. B., K. E. RUSSELL, AND J. B. WILSON. 1963. Comparison of two media for the detection of the egg yolk factor of *Staphylococcus aureus*. J. Bacteriol. **85**:1181–1182.
32. SHAH, D. B., AND J. B. WILSON. 1963. Egg yolk factor of *Staphylococcus aureus*. I. Nature of the substrate and enzyme involved in the egg yolk opacity reaction. J. Bacteriol. **85**:516–521.
33. SMUCKLER, S. A., AND M. D. APPLEMAN. 1964. Improved Staphylococcus Medium No. 110. Appl. Microbiol. **12**:355–359.
34. SUBCOMMITTEE ON TAXONOMY OF STAPHYLOCOCCI AND MICROCOCCI. 1965. Minutes of first meeting. Intern. Bull. Bacteriol. Nomen. Taxon. **15**: 107–108.
35. WILLIS, A. T., AND G. C. TURNER. 1962. Staphylococcal lipolysis and pigmentation. J. Pathol. Bacteriol. **84**:337–347.