THE BACTERIOLOGY OF ERYSIPELOID

BY

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The published descriptions of *Erysipelothrix rhusiopathiae* vary a good deal, and there is no recent full comparison of human strains with those from animals. We therefore examined in detail, including serology and sensitivity to antibiotics, the seven strains isolated from the patients with erysipeloid whose case histories are described by Price and Bennett (1951) at page 1060 of this issue and compared them with strains from other sources.

Sources of Material.—*Human strains*: Seven strains isolated by us (K13, K19, K45, K49, K53, K79, and K176) and two by Dr. Mary Barber (9 and 21). *Animal strains*: Seven received from Dr. A. W. Gledhill (D, R, V, EW2, EW16, EW24, and MEW22) and two from Dr. D. Riding (PV174 and SE46).

Method of Isolation

Biopsy specimens from the skin of 20 patients were cultured. The specimens were taken from the spreading edge of the erysipeloid and included the entire thickness of the dermis; they were placed whole in 1% glucose broth and incubated aerobically at 37° C. for one week. The broth was subcultured aerobically and anaerobically every other day on blood-agar plates. All broths yielding *E. rhusiopathiae* became turbid within 48 hours. The strains isolated from the seven positive skin samples were preserved by freeze-drying within a few days of isolation.

We examined these strains in detail together with Barber's two human strains (9 and 21) and two veterinary strains (D, isolated from a duck, and PV174, from swine erysipelas). There were no appreciable differences as regards microscopical or colonial morphology or biochemical activity among any of these strains, except that PV174 grew rather more slowly than the others.

Description of our Strains

Morphology on Horse-blood Agar.—Small slender rods about 0.4 μ by 2.5 μ in size, straight or slightly curved, single, and forming unbranched chains or filaments up to about 20 μ long. In old cultures longer forms predominated. Gram-positive, evenly staining but easily decolorized; not acid-fast, non-motile, forming no spores. No capsules detected. All the strains were smooth colonially, but the microscopical appearance, even on primary isolation, was closer to the "intermediate" type described by Barber (1939), consisting mainly of chains of medium length and few single bacilli.

Cultural Appearances

Horse-blood-agar Plate.—Aerobically; 37° C. *Twenty-four hours*: Small round, glistening, transparent colonies about 0.2 mm. in diameter. *Forty-eight hours*: Colonies 0.6 mm. in diameter, round, low convex; surface smooth and shiny, with entire or slightly undulate edge and surrounded by a narrow zone of alpha-haemolysis; butyrous

and easily emulsified. Agar pitted on removal of colony. *Seven days*: Colonies 2 mm. in diameter, flattened, with a low central papilla, radial striations, and a lobate edge. Haemolysis and dark central pigment well marked. Colonies grown anaerobically very similar in shape and size.

Nutrient-agar Plate.—Colonies similar to those on blood agar, but a little smaller. Central patches of brown pigmentation visible at 48 hours and more pronounced after further incubation.

Gelatin Stab at 22° C.—*Twenty-four hours*: Faint hazy growth a few millimetres below the surface. *After a few days* the constant and characteristic feature was the column of uniform diameter running from top to bottom of the medium, composed of fine horizontal outgrowths from the stab line (the classical "lampbrush"). No liquefaction within 14 days. The swine strain PV174 differed from all other tested strains by growing poorly in the depth.

Broth.—Slight turbidity with powdery deposit after 24 hours, easily broken up. Deposit stringy after a few days. No pellicle. Odour characteristic and suggestive of stewed beef. The addition of 2% serum or 0.2% glucose to broth gave rather better growth.

1% Glucose-agar Shake.—37° C. *Twenty-four hours*: Uniform growth of minute discrete colonies throughout the medium.

Other Tests

Biochemical.—The reactions of all 11 strains were identical when tested with 1% sugar in 1% peptone. Acid, without gas, was formed in glucose, lactose, fructose, galactose, and mannose; in xylose a very small amount of acid was produced. No acid production was detected after 14 days' incubation in maltose, sucrose, mannitol, dulcitol, salicin, arabinose, rhamnose, adonitol, inositol, erythritol, dextrin, starch, glycogen, inulin, amygdalin, aesculin, raffinose, cellobiose, melibiose, melezitose, trehalose, sorbose, and sorbitol. A trace of acid but no clot in litmus milk, Methyl red, Voges-Proskauer, and tests for indole and ammonia, for nitrate and methylene-blue reduction, for hydrolysis of sodium hippurate, and for urease and catalase were negative. A moderate degree of phosphatase activity was detected (Bray and King, 1943). No growth in Koser's citrate. All strains produced H₂S.

Metabolism.—Growth was approximately equal under aerobic and anaerobic conditions, and was not appreciably affected by the addition of 10% CO₂ in either atmosphere. The optimum temperature for surface growth on blood agar is 30° C. to 40° C. Growth occurred in broth from pH 6.7 to 9.2, with an optimum at pH 8 to 8.5. In peptone water, growth was invariably scanty. No soluble haemolysin for horse, sheep, human, rabbit, or guinea-pig red cells.

Pathogenicity.—All our strains killed mice within four days of a subcutaneous inoculum of 0.01 ml. of a 24-hour broth culture. The mice developed a typical conjunctivitis, and post-mortem cultures from heart blood and spleen were positive in every case.

Resistance.—Broth cultures were killed at 60° C. within five minutes. When *E. rhusiopathiae* was heavily inoculated into broth containing 10% sodium chloride the cultures became sterile within 24 hours at 37° C.

Sensitivity to Dyes and Antibiotics

In view of the possible uses of selective media, nutrient agar containing tenfold dilutions of various bacteriostatic substances, from 1:1,000 to 1:1,000,000, was lightly inoculated and incubated at 37° C. for 24 hours. The behaviour of each of the 11 strains tested was identical; the results are shown in Table I.

All strains grew scantily on MacConkey's agar in 48 hours, and not at all on potato or Hoyle's tellurite agar.

TABLE I.—Effect of Various Bacteriostatic Substances on Growth of *E. rhusiopathiae*

	Concentration Completely Inhibiting Growth	Concentration Permitting Free Growth
Sodium azide	>1:1,000	1:10,000
Potassium tellurite	1:100,000	<1:1,000,000
Sodium biselenite	1:1,000	<1:1,000,000
Methylene blue	1:10,000	<1:1,000,000
Thionine	1:1,000	1:1,000,000
Basic fuchsin	1:1,000	1:100,000
Pyronine	>1:1,000	1:10,000
Crystal violet	1:10,000	1:1,000,000
Brilliant green	1:100,000	<1:1,000,000
Acriflavine	1:100,000	1:1,000,000
Cetyl ammonium bromide (cetrimide)	1:100,000	1:1,000,000

The sensitivity to various antibiotics (Table II) was tested as follows: varying amounts of antibiotic were added to nutrient agar (pH 7.4–7.6) at 50° C.; plates were poured, dried, and inoculated over an area the size of a sixpence with a loopful of a 1:10 dilution of an overnight broth culture of the organism to be tested.

TABLE II.—*In vitro* Sensitivity of *E. rhusiopathiae*

Chemotherapeutic Agent	Range of Inhibitory Concentration for <i>E. rhusiopathiae</i>
Sulphathiazole	>5 mg./100 ml.
Penicillin	0.06–0.12 unit/ml.
Streptomycin	12.5–50 µg./ml.
Aureomycin	1.5–2.5 µg./ml.
Chloramphenicol	5–9 µg./ml.
Polymyxin E	>80 µg./ml.

Strains tested: K13, K19, K45, K49, K53, K79, K176, 9, 21, PV174, R, D, MEW22, EW2, EW24, EW16.

Results were read after 24 hours' aerobic incubation at 37° C. The *in vitro* sensitivity to penicillin, chloramphenicol, and "aureomycin" was such that all strains fell within the therapeutic range of these drugs; all, however, were resistant to the usual therapeutic blood-concentrations of sulphathiazole and polymyxin E. There were considerable individual variations in the degree of resistance to streptomycin; some strains, however, were susceptible to concentrations equal to those obtainable in the blood.

These findings are in accordance with therapeutic experience: thus many workers—for example, Klauder and Rule (1944), Barber, Nellen, and Zoob (1946), and King (1946)—found sulphonamides useless in the treatment of human erysiploid, and Klauder and Rule (1946) concluded that streptomycin had little effect in experimental mouse infections. On the other hand, successful therapeutic responses to penicillin are now well known in both human and experimental disease, and the observations of our colleagues (Price and Bennett, 1951) bring further evidence on this point. In view of the *in vitro* tests both aureomycin and chloramphenicol would seem worthy of clinical trial, though in the treatment of experimental infections in birds Prier and Alberts (1950) reported penicillin to be more effective than aureomycin.

Antigenic Structure

From the work of Watts (1940) and Gledhill (1945) it appears that the species has several thermostable O antigens, which are distributed in varying amount among the individual members of the species, and that every strain is agglutinated by antiserum prepared against any other strain. All our strains were agglutinated to titre by a polyvalent swine erysipelas antiserum. By cross-absorption Gledhill (1945) has distinguished several antigenic groups in his strains of

animal origin. We immunized rabbits with boiled organisms of his Groups I, II, III, and IV, and prepared group-specific sera by absorption with heterologous strains. Unfortunately the titres fell very considerably as a result of absorption.

Table III shows the results of testing these sera against our strains by Gledhill's methods. As saline suspen-

TABLE III.—Agglutination of Individual Strains of *E. rhusiopathiae* by Group-specific Sera

Strains	Titres to Antisera				Pre-sumed Group
	Group I Anti-D Absorbed c EW2 (II) and EW16 (III)	Group II Anti-EW2 Absorbed c D(U) and EW16 (III)	Group III Anti-EW16 Absorbed c D(U) and EW2 (II)	Group IV Anti-MEW22 Absorbed c D(U) and EW16 (III)	
Type strains:					
Group I { D ..	160	20	—	—	
R ..	40	—	—	—	
" II { EW2 ..	—	40	—	—	
EW24 ..	—	80	—	—	
" III { EW16 ..	—	—	80	—	
V ..	—	—	40	—	
" IV MEW22 ..	—	—	—	40	
Unknown strains:					
{ K13 ..	80	—	—	—	I
K19 ..	80	—	—	—	I
K45 ..	—	—	20	—	III
K49 ..	—	—	—	—	Untyp- able
Human { K53 ..	80	80	—	—	I+II
K79 ..	160	—	80	—	I+III
K176 ..	—	—	—	—	Untyp- able
{ 9 ..	—	—	40	—	III
21 ..	20	20	—	—	Doubt- ful
Porcine { PV174 ..	40	—	—	—	I
SE46 ..	—	—	80	—	III

Titre expressed as reciprocal of serum dilution. — = less than 20.

sions tend to be unstable and frequently show fine degrees of agglutination, almost complete agglutination was taken as the end-point. Two of our strains appeared to fall into Gledhill's Group I, one into Group III, one into Group I+II, and one into Group I+III; two we were unable to type. Gledhill found that about one-third of his strains were of Group I, one-sixth of Group II, one-sixth of Group III, the remainder being of other types, such as IV and I+II, or untypable. Thus the human strains are distributed amongst the same groups as animal strains.

Discussion

Though easily cultivated when once isolated, there is general agreement that *E. rhusiopathiae* is not easily grown from human erysiploid by the usual methods of sampling; for successful isolation it would seem necessary to perform a biopsy which includes the whole thickness of the skin (Düttmann, 1921; Barber, Nellen, and Zoob, 1946). In our series 7 out of 20 biopsy specimens were positive for *E. rhusiopathiae*; several cultures remained sterile; in the majority of positive isolations growth was obvious within 24 hours (Table IV).

The explanation of our failure to isolate erysiplothrinx from a higher percentage of specimens is not clear, but may be due to: (1) Patchy or scanty distribution of erysiplothrinx in the tissues. In a recent isolation the organism was grown only from a second biopsy. In this case it is suspected that the first biopsy had not been taken from the active spreading edge of the lesion.

TABLE IV.—Results of Culture from Skin of 20 Patients Diagnosed Clinically as Erysipeloid

Case No.	<i>E. rhusiopathiae</i>	Other Organisms
1	+	—
2	—	Aerobic spore-bearing bacillus
3	+	—
4	—	—
5	—	<i>Cl. welchii</i>
6	—	—
7	+	—
8	+	Coagulase-negative staphylococcus
9	+	—
10	—	Coagulase-negative staphylococcus
11	—	" " "
12	—	—
13	+	Coagulase-negative staphylococcus
14	—	—
15	—	—
16	—	Coagulase-negative staphylococcus
17	—	Coliform bacillus
18	—	—
19	+	—
20	—	—

It may be noted also that the length of clinical history before biopsy varied from a few days to several weeks, and that three patients had received penicillin before biopsy. (2) Unsuitability of the medium for certain fastidious strains. (3) Inability of the organism to grow out from the centre of a dense piece of tissue. (4) Inhibitory effect of cetrimide used in cleaning the skin, or of local analgesic solutions. (5) The lesions sampled were manifestations of diseases of different aetiology but similar clinical features.

From the data of our colleagues Price and Bennett (1951) there are grounds for considering that this last explanation is unlikely. The other organisms isolated from our biopsy material certainly are not likely to have any aetiological importance; coagulase-negative staphylococci, the commonest of skin saprophytes, were recovered in five instances, and *Clostridium welchii*, aerobic spore-bearing bacilli, and coliform bacilli were each found on one occasion; *Cl. welchii* is well recognized as one of the most ubiquitous of anaerobic bacteria.

The presence of these organisms in our cultures, however, may have adversely affected our results, since in a fluid medium contaminants are apt to overgrow erysipelotheix. The use of a selective medium might increase the proportion of isolations. For this reason we tested the effect of various bacteriostatic substances (Table I). In its sensitivity to dyes *E. rhusiopathiae* resembles most Gram-positive organisms, but is similar to the haemolytic streptococcus in its comparative resistance to crystal violet. Our strains grew well on blood agar at pH 6.8 containing 1:1,000 sodium azide and 1:100,000 crystal violet (Packer, 1943).

In view of the long survival of this organism in salted meat (Edwards, 1931) it is perhaps surprising that it dies so rapidly in broth containing 10% sodium chloride. The salt concentration, however, is seldom uniform throughout a piece of pickled meat, and varies very greatly according to the method of salting.

There is much confusion in the literature about the fermentation reactions of erysipelotheix. We believe that much of it is due to the small amount of acid produced, even in glucose; results may vary with the indicator and composition of the medium. We obtained doubtful reactions with one batch of maltose, arabinose, and cellobiose, in which all the strains produced a trace of acidity: on repetition with fresh batches the reactions were all negative. Observations by Woodbine (1950) also suggest that many discrepancies in fermentation

reactions may be due to differences in the medium employed; he finds that, whereas maltose is invariably fermented in the presence of 5% bovine serum, no fermentation of this sugar occurs in unenriched peptone water, and that if horse serum is used maltose fermentation is variable. We have not had an opportunity of confirming this statement, as we used unenriched peptone water for all our tests. We cannot, however, confirm Woodbine's failure to obtain growth in litmus milk. Hutner (1942) has shown that the growth of erysipelotheix in simple media is greatly improved by traces of oleic acid and riboflavin; it is possible that variations in the amounts of these substances in different peptones may account for the conflicting reports concerning the ability of erysipelotheix to grow in peptone water. The results obtained in testing for hydrogen sulphide may likewise vary with the medium or method used. All our strains produced hydrogen sulphide in some quantity, as shown when screw-capped agar slope cultures were tested by lead-acetate paper. In general our findings are in good agreement with the more extensive studies of Deem and Williams (1936), Karlson (1938), Barber (1939), and Julianelle (1941).

In the erysipelotheix group three species were differentiated by Rosenbach (1909)—*murisepticus*, *porci*, and *erysipeloides*, those isolated from mouse septicaemia, swine erysipelas, and human erysipelotheix respectively. Our failure to find any morphological, cultural, or biochemical differences between human strains and those isolated from animals merely adds further weight to the opinion first advanced by Rickmann (1909) and supported by many later workers that there is insufficient evidence to warrant this classification, and we follow the practice of Topley and Wilson's *Principles of Bacteriology and Immunity* (1946) in contradistinction to that of Bergey's *Manual* (1948) in regarding the three organisms as belonging to one species, *E. rhusiopathiae*.

There is evidence, however, that there are considerable strain differences in virulence; thus, whereas pigs are susceptible to inoculation with the organisms isolated from acute swine erysipelas, attempts to infect them with erysipelotheix isolated from an epizootic in lambs (Cornell and Glover, 1925) were unsuccessful; lambs which are resistant to infection with virulent porcine organisms were readily infected with this strain. Similarly, field mice, which are also said to be normally insusceptible to erysipelotheix infections, have been the subject of a widespread epizootic in California (Wayson, 1927). We have not made a careful study of the pathogenicity of the organisms we isolated; all, however, were highly virulent to laboratory mice.

The Relation of Human to Animal Infections

As to the virulence of animal strains for man, little direct evidence exists. Rosenbach (1887) and Ohlemann (1904) developed erysipelotheix after inoculating their forearms with cultures of erysipelotheix, but both cultures were ones which had previously been isolated from human lesions. However, Rahm (1919), in discussing the aetiology of human erysipelotheix, refers to numerous infections among veterinary surgeons following needle pricks from syringes containing living cultures and among laboratory workers from broken culture tubes. These cultures would almost certainly have been porcine in origin.

Erysipelotheix has for many years been recognized in Europe and America as an occupational disease of

abattoir employees, butchers, kitchen workers, and those handling fish; Klauder (1938), in reviewing 100 cases, was able to obtain a history of contact with animals, fish, or animal products, such as hides, in nearly every case. Epidemiological evidence therefore strongly suggests that man becomes infected through contact with animals or animal material, though in most cases, it must be admitted, the infection of this material has been inferred rather than proved. The relative infrequency of erysipeloid in those tending sick pigs (Bierbaum and Gotttron, 1929) is surprising, for it would be thought that farm workers and veterinarians would be as liable to trauma as, say, butchers or workers in a button factory. The prolonged survival of virulent organisms in inanimate material; the natural history of outbreaks of swine erysipelas, pointing to soil infection rather than to spread from other centres of infection (Edwards, 1931); and the independent infections of other animals—for example, sheep and field mice—with organisms having a specially high virulence for that species, have led many to suggest that erysipelotheix may lead a saprophytic existence outside the body.

McGinnes and Spindle (1934), in investigating the erysipeloid epidemic among workers in a bone-button factory, noted that the incidence was highest among those who were not only liable to traumatic injury but also working under moist conditions; and in the well-known Odessa epidemic, in which about 200 cases of erysipeloid occurred within two months of the landing of large quantities of a fish of the perch-pike species, Stefansky and Grunfeld (1930) noted that the lesions were typically preceded by a wound from the teeth or fins of the fish during cleaning; again conditions would be moist and, as such, favourable to the spread of saprophytic organisms. The fish appeared healthy but were not examined bacteriologically. Schoop (1936), in Germany, and various workers in Japan, however, have since reported erysipelotheix infections in mice inoculated with slime from the surface of healthy fish of various species.

Evidence, however, is accumulating that *E. rhusiopathiae* may be more widely distributed in the animal kingdom than was formerly thought. It is said to occur fairly commonly in birds of most species (Gledhill, 1948), and, in reviewing the literature, Woodbine (1950) refers to reports of isolated infections in the kangaroo, dog, horse, wild boar, reindeer, and mink; he considers, however, that natural infection in fish has yet to be proved. Of considerable importance, perhaps, are the very occasional reports of infection in rats (Crougue, 1936; Stiles, 1944; Drake and Hall, 1947), because if, as Drake and Hall suggest, erysipelotheix infections in the brown rat are more common than supposed, many puzzles in the epidemiology of erysipelotheix infections may be solved.

Our antigenic studies are by no means complete, but we could recognize certain major antigenic components in our organisms and assign many of them to Gledhill's groups. Though the numbers are small, it is, we think, significant that the strains recovered within the space of a few months from patients with human erysipeloid in a small area of London are distributed amongst the same groups as Gledhill's veterinary strains. Thus our studies emphasize once again how closely related are human strains to those found in animals, and they provide no reason for assuming that human strains are peculiarly virulent for man.

Summary

Erysipelothrix rhusiopathiae was isolated from seven out of twenty patients with erysipeloid.

The seven strains were culturally and biochemically homogeneous and indistinguishable from veterinary strains.

They were distributed among several antigenic groups, and were relatively uniform in their sensitivity to each of various antibiotics. They were most sensitive to penicillin, aureomycin, and chloramphenicol.

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The new factory of Ethicon Suture Laboratories, Ltd., opened at Fountainbridge, in Edinburgh, on October 19, cost about £30,000 to build and equip, and employs 58 men and 131 women. By 1939 approximately half the sutures used in Britain were made in the country, either at Edinburgh or in other factories, and the other half were imported from America. During the war great progress was made by the industry, and at the end of the war Britain was almost self-sufficient so far as sterilizing was concerned, but 50% of the raw strings had to be imported. It is estimated that Britain will now be able to produce all the cat-gut strings used in our hospitals. The raw material used in the manufacture of sutures is the upper part of the intestine of sheep. The first operation at the factory is that of washing and opening out the hanks, which are then split into ribbons and processed to remove all mucus. The gut is next spun into strings, which are dried, polished, and gauged. The laboratory controls all these processes, and after sterilization further tests are made before the suture is finally packed ready for use. The new factory will house the full "raw" process, and only the final work will be done at the Sighthill factory.