This histological finding may have a definite diagnostic value, but more cases are required to confirm this.

Table VI shows how the diagnoses in group I were reached. Of 30 patients who were proved to have a malignant intrathoracic growth associated with a pleural effusion biopsy was negative in 10. Thus no reliance can be placed on negative biopsies; a plaque of growth on the pleura is easily missed in a blind biopsy. From our small series it would appear that in pleural effusions associated with intrathoracic malignant disease biopsy will give a false negative result in about half the cases.

In tuberculous effusions the picture is different. In group I we had 73 such cases, and in only 3 was biopsy negative and bacteriological examination positive. Of the 70 in whom biopsy was positive, bacteriology was negative in 45. There were 12 further patients in this group in whom the effusion was probably tuberculous. If we accept 85 as the total number, then in tuberculous pleural effusion biopsy will be positive four times out of five. The chances of obtaining a positive biopsy are uninfluenced by the size and white-cell count of the effusion, the presence of parenchymal lung shadows, the antituberculous chemotherapy already given (though we have not had sufficient experience with cases treated for more than six weeks), and the duration of the symptoms.

Because of the wide variety of diseases in groups II and III, an overall analysis was not worth while.

In 1 patient with sarcoidosis, the pleura was normal. Another patient, diagnosed on scalene-node biopsy, developed a small pleural effusion six months later. Biopsy of the pleura showed non-specific fibrous thickening. Of 6 patients with reticulosis and pleural effusion 1 was shown by biopsy to have a lymphocytic tumour involving the pleura. 2 solid tumours have been biopsied with the needle—a giant-cell tumour of bone lying deep to the pleura and an osteosarcoma of rib.

Jacobæus (1911, 1925) and Unverricht (1923) described tuberculous granulation tissue in both pleural membranes in cases of tuberculous effusions. Table VII summarises published data of later workers. Donohoe et al. (1958) believe that a non-diagnostic needle biopsy has no value, and that it should always be followed by a full surgical biopsy. Our needle is probably technically superior, and negative biopsies have had at any rate some significance in our series. We feel, however, that needle biopsy will not altogether eliminate the need for surgical biopsy, and that the latter should be considered where other methods have failed.

Donohoe et al. (1958) found non-specific inflammatory changes in 21 of their 54 surgical biopsy specimens: and in only 5 of them was a definite diagnosis (tuberculosis) afterwards proved. They suggest that there exists a "non-specific pleuritis", possibly a virus infection, which is akin to non-specific acute pericarditis and which consists of a transient acute effusion. 2 cases of "fibrinous pleuritis" described by Douglass et al. (1956) were possibly examples of the same condition; and we have seen 2 that may also fall into this category.

Conclusions and Summary

We report our experience with blind punch biopsy of the parietal pleura in 200 patients.

The method is simple and safe. Since it can establish the diagnosis in about 80% of tuberculous and 60% of malignant effusions, it is by far the most reliable diagnostic technique when tuberculosis is suspected.

We have had only 1 false diagnosis in 228 biopsies.

This procedure may be the only way of making a definite diagnosis during life. It should be part of the initial investigation of every patient with a pleural effusion of uncertain ætiology.

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IN-VITRO ACTIVITY OF RISTOCETIN AND FRAMYCETIN

TWO NEW ANTIBIOTICS

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As the search for new antibiotics continues, it is important that the in-vitro activity of each new product should be carefully determined in order to obtain some knowledge of its therapeutic possibilities. We have consequently investigated the in-vitro activity of two recently introduced antibiotics, ristocetin and framycetin.

Ristocetin ('Spontin' Abbott) is a new narrow-range antibiotic, isolated from cultures of a species of Actinomycete, *Nocardia lurida*; it has two components, ristocetin A and B, which have a similar range of activity against gram-positive bacteria and mycobacteria (Grundy et al. 1958).

Framycetin is an antibiotic produced by a strain of *Streptomyces decaris* (R.2103) and is available as the sulphate salt ('Soframycin' Roussel). A wide range of activity is claimed, including *Staphylococcus aureus*, *Pseudomonas pyocyanea* and the proteus group (Massénat-Déroche 1954).

Both ristocetin and framycetin are freely soluble in water and are stable for at least four months when stored in aqueous solution (20,000 μg. per ml.) at 4°C.

Methods

The sensitivity of freshly isolated common pathogens to the two antibiotics was assessed by our standard serial dilution technique (Fairbrother and Williams 1956). A standard drop (0·02 ml.) of an undiluted overnight broth culture was added to 0·5 ml. of nutrient broth or serum broth containing doubling dilutions of the antibiotic, and the results were read after 18 hours' incubation at 37° C.

Results

Ristocetin

140 organisms were tested against ristocetin by the serial dilution technique, and the results show particular activity against gram-positive cocci with little effect against gram-negative organisms (table I). The pneumococcus was the most sensitive organism; all strains were inhibited by 2 μg. per ml.; *Str. pyogenes* (group A) was

TABLE I—SENSITIVITY TESTS WITH RISTOCETIN AND FRAMYCETIN BY THE SERIAL DILUTION TECHNIQUE

Minimum inhibitory concentration (μg. per ml.)	*Staph. aureus*		*Str. pyogenes*		*Str. fæcalis*		Pneumococcus		Coliforms		Proteus		*Ps. pyocyanea*	
	R	F	R	F	R	F	R	F	R	F	R	F	R	F
2	..	..	3	..	1	..	5	..	..	..	..	..	..	1
4	..	6	10	..	8	..	..	5	..	..	..	..	..	1
8	4	8	..	..	5	..	..	..	..	2	..	..	..	3
16	37	25	..	..	..	..	..	..	..	7	..	1	..	6
32	9	9	..	2	..	..	..	..	..	11	..	5	..	4
64	..	2	..	4	..	2	..	..	..	16	..	15	..	6
128	..	..	..	3	..	3	..	..	..	7	..	4	..	2
256	..	..	..	4	..	9	..	..	..	2	..	1	..	..
>256	..	..	..	..	..	..	..	..	25	..	16	..	17	..
Total	50	50	13	13	14	14	5	5	25	45	16	26	17	23

R=ristocetin. F=framycetin.

also very sensitive, 3 of 13 strains were inhibited by 2 μg. per ml. and the remaining 10 strains were inhibited by 4 μg. per ml. *Str. fæcalis* was also sensitive; none of the 14 strains examined required more than 8 μg. per ml. for inhibition of growth.

Staph. aureus was relatively resistant, and of 50 strains only 4 were inhibited by 8 μg. per ml.; the majority (37) required 16 μg. per ml., while 9 required 32 μg. per ml., to inhibit growth.

Gram-negative bacilli proved resistant and were not inhibited by at least 256 μg. per ml.

Framycetin

176 organisms were tested against framycetin (table I). The results show a wide range of activity against both gram-positive and gram-negative organisms, with, in some cases, considerable variation in sensitivity between different strains of the same organism. The pneumococcus proved the most sensitive organism; all strains were inhibited by 4 μg. per ml. Strains of *Staph. aureus* showed wide differences in their sensitivity. Of 50 strains 6 were inhibited by 4 μg. per ml.; but 25 were relatively resistant, requiring 16 μg. per ml. to inhibit growth; whilst 2 required 64 μg. per ml. *Str. pyogenes* (group A) and *Str. fæcalis* showed a similar pattern, and were both moderately resistant; the 13 strains of *Str. pyogenes* required 32–256 μg. per ml. for inhibition and the 14 strains of *Str. fæcalis* were only inhibited by 64–256 μg. per ml.

Framycetin showed very variable activity against the gram-negative organisms; 5 of 23 strains of *Ps. pyocyanea* could be classified as sensitive, requiring 8 μg. per ml. or less to inhibit growth; of the remaining 18 strains 2 required as much as 128 μg. per ml. to inhibit growth.

The average minimum inhibitory concentration (M.I.C.) for coliforms and proteus was 64 μg. per ml., but occasional strains required 256 μg. per ml.—which exceeds practical levels attainable in the tissues.

Development of Resistance

Framycetin.—It has been claimed that resistance in-vitro to framycetin is slow to develop (Massénat-Déroche 1954), but we have not been able to confirm this. Strains of *Staph. aureus* and *Ps. pyocyanea* were grown in nutrient broth containing antibiotic, as in the serial dilution technique. After 18 hours' incubation at 37°C, the M.I.C. was read, and the broth containing the highest concentration of antibiotic permitting growth was used as the inoculum for a second series. Passages were continued in this way, and the M.I.C. was recorded 18 hours after each passage. The results (table II) show that in-vitro resistance to framycetin develops rapidly; at the third passage, the three strains of *Staph. aureus* investigated showed a threefold increase in resistance; at the twelfth passage, two of the strains showed an eightfold increase in resistance and required more than 1024 μg. per ml. for inhibition of growth. *Ps. pyocyanea* showed an even more rapid development of resistance; at the third passage the five strains investigated showed threefold to sixfold increase, and at the sixth passage three of the strains showed sixfold to eightfold increase in resistance. One strain acquired an eightfold increase in resistance at the ninth passage. In all cases the experiment was terminated when the M.I.C. was greater than 1024 μg. per ml.

Ristocetin.—A similar experiment was conducted with ristocetin using the same three strains of *Staph. aureus* and one strain of *Str. fæcalis*. The results (table II) show

TABLE II—ACQUIRED RESISTANCE IN VITRO TO FRAMYCETIN AND RISTOCETIN

No. of passage	Fold increase in resistance											
	Framycetin								Ristocetin			
	Staph. aureus (1)	*Staph. aureus* (2)	*Staph. aureus* (3)	*Ps. pyocyanea* (1)	*Ps. pyocyanea* (2)	*Ps. pyocyanea* (3)	*Ps. pyocyanea* (4)	*Ps. pyocyanea* (5)	*Staph. aureus* (1)	*Staph. aureus* (2)	*Staph. aureus* (3)	*Str. fæcalis*
3rd	3	3	3	6	6	5	5	3	0	0	0	2
6th	4	4	4	..	..	8	7	6	2	0	2	2
9th	6	5	5	..	..	..	..	8	2	2	2	2
12th	..	8	8	..	..	..	..	..	2	2	2	2

only a twofold increase in resistance after twelve passages, with all strains.

Comments

Ristocetin

Our results indicate that, while ristocetin has a similar range of antibacterial activity to penicillin, erythromycin, bacitracin, and vancomycin, its clinical application is likely to be limited. It has to be given intravenously and therefore its administration presents difficulties; moreover it exhibits only moderate antibacterial activity. It may, however, prove useful in the treatment of infections due to antibiotic-resistant *Staph. aureus* and of enterococcal endocarditis; several such cases have been treated successfully (Taylor et al. 1958, Romansky and Holmes 1958). Disadvantages are associated with the administration of ristocetin; following its use minor reactions, such as rashes, thrombophlebitis at the site of injection, neutropenia and thrombocytopenia have been reported (Gangarosa 1958).

Framycetin

Framycetin has a wide range of antibacterial activity, but cannot be given parenterally as it is highly toxic to the kidneys and the eighth cranial nerve. It is usually applied topically in the form of an ointment containing 1·5% framycetin sulphate in a water-miscible base, and good results have been claimed in cases of superficial skin infections, especially those due to *Staph. aureus* (Burrows 1958). It should prove useful also in the treatment of superficial infections due to the more sensitive strains of *Ps. pyocyanea* and proteus as relatively high concentrations can be applied. It has also been used orally, with good results and absence of toxicity, in cases of infantile gastroenteritis due to pathogenic *Escherichia coli* (Louwette and Lambrechts 1958).

In-vitro tests, however, suggest that resistance can develop rapidly and caution will have to be exercised in its general use. Also, the wide variation in sensitivity between different strains of the same organism necessitate critical in-vitro assessment of the sensitivity of the infecting organism in each individual case.

Conclusion

Sensitivity tests by the serial dilution technique indicate that ristocetin is effective against gram-positive cocci, and that framycetin is moderately effective against both gram-positive and gram-negative organisms.

Both antibiotics appear to have limited clinical applications and certain disadvantages, and there seems to be little indication for their general use in routine practice, although framycetin should be valuable for the local treatment of some superficial infections. Careful laboratory control is necessary.

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FILTERED LIQUID PLASMA FOR TRANSFUSION

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THE transfusion of unmodified human blood-plasma is associated with the risk of transmitting homologous-serum jaundice. Various methods of processing the plasma in order to inactivate the virus of this disease have been investigated and have resulted in products such as the following:

(1) Purified human albumin (Cohn et al. 1946), heat-treated in solution for 10 hours at 60°C in the presence of protein stabilisers.

(2) Crude albumin preparations which have been similarly heat-treated. These are obtained by precipitating the fibrinogen and part of the globulins from the plasma by the addition of zinc salts (Surgenor 1952), by alcohol fractionation (Mulford et al. 1955, Hink et al. 1957, Krijnen and Mastenbroek 1957), or by desalting with ion-exchange resins (Nitschmann et al. 1956).

(3) Plasma treated with β-propiolactone and irradiated with ultraviolet light (Hartman and LoGrippo 1957).

(4) Liquid plasma stored at 30–32°C for six months prior to use (Allen et al. 1954).

Of these, the last-named product is the simplest to prepare and is giving promising results from the point of view of virus inactivation (Allen et al. 1954). It has the serious disadvantage, however, that in the cloudy final product the visual detection of bacterial contamination is difficult.

In this laboratory a technique has been worked out for the preparation of a treated liquid plasma which does not have this disadvantage. The method makes use of the kaolin-treatment procedure of Maizels (1944), which permits the subsequent filtration of the plasma through asbestos pads. In the resulting clear liquid, bacterial contamination gives rise, after incubation, to opacity which is obvious on inspection. The clear plasma obtained by a single kaolin treatment is not stable, and a flocculent protein precipitate appears after it has stood at room temperature or at 32°C for two to six months.

Maizels observed that a second kaolin treatment, carried out after storing the plasma for six months or a year at room temperature, resulted in a clear product which was stable for at least two years. In the present investigation re-treatment with kaolin after storage for six months at the higher temperature of 32°C was therefore tried, to see whether a similar stable liquid product could be obtained.

Method

Pools of liquid plasma (1800 ml.), obtained in the usual way from outdated blood which has been collected in standard acid-citrate-dextrose anticoagulant, are mixed with 120 g. kaolin in sterile Winchester bottles. After samples for sterility testing have been removed the bottles are shaken, frozen, and thawed. The plasma is decanted from the sedimented kaolin, pooled, filtered through clarifying and sterilising pads, and collected in sterile bottles. The sterility of each is then established by inspection after lengthy incubation both at room temperature and at 32°C. Storage at the latter temperature is continued for six months to inactivate the hepatitis virus. During this period some flocculent protein is precipitated.

At the end of the six months' storage the plasma is again examined for signs of bacterial contamination. It is then mixed with a further quantity of kaolin, shaken mechanically for an hour, frozen, thawed, filtered through clarifying and sterilising pads, and distributed in 400 ml. quantities. All the final containers are incubated at room temperature and then at 32°C, and inspected for signs of bacterial growth or moulds, before being released for clinical trial. The procedure is in addition controlled throughout in the usual way by bacteriological cultures on representative samples.

The liquid plasma thus obtained remains clear and translucent, except for a very slight trace of flocculent precipitate which appears shortly after the final filtration. This flocculation is too slight to interfere with detection of bacterial contamination by naked-eye inspection, nor has it increased appreciably on storage for a further nine months. The product contains 3·5–4% protein and has a pH of 6·5–7·0. The loss of plasma by occlusion in the sedimented kaolin totals about 12% by volume.

Comment

In preliminary clinical trials, 5·2 litres of the material have been transfused into one patient over a period of six weeks, without adverse reaction or any clinical evidence of sensitisation.

The possibility of preparing the same material with a final protein content of 7·5% is under investigation. This would correspond more closely to the normal " in-vivo " plasma-protein content. The product may be freeze-dried, and on reconstitution gives a solution which is only slightly cloudy.

This method seems to be promising from the points of view of virus inactivation, safety, and economy; because the product is in liquid form it is convenient to handle. This plasma is deficient in fibrinogen and other clotting factors, and is therefore unsuitable for patients specifically requiring these substances.

References overleaf