THE *IN VITRO* DIFFUSION OF ANTIBIOTICS THROUGH FIBRIN MEMBRANES

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IT is well known that the host-parasite relationship in certain infections due to various micro-organisms is such that there is little stimulation of antibacterial immunity. This applies in particular where the organisms are confined within the initial site of infection by the deposition of fibrin barriers in the connective tissues. Previous work by Hughes (1948) has demonstrated that membranes prepared from human fibrin have a selective effect with regard to the passage of various dyes, toxins and proteins. Comparatively little attention has been paid, however, to the problem of what effect fibrin barriers have on the diffusion of antibiotics. One of the few studies is that of Weinstein, Daikos and Perrin (1951) who showed that penicillin is able to diffuse freely into fibrin clots implanted subcutaneously in rabbits.

The present investigation was undertaken in an attempt to determine whether fibrin membranes would exert a selective action on the passage of a number of antibiotics *in vitro* since the rational use of antibiotic therapy in infections characterized by fibrin deposition will obviously be determined to some extent by the ability of the drugs to penetrate the fibrin barrier.

MATERIALS AND METHODS

Antibiotic stock solutions.—Stock solutions of crystalline penicillin G (20,000 units/ml.), chloramphenicol (1,000 μg./ml.) and chlortetracycline (1,000 μg./ml.) were prepared in sterile 0·85 per cent saline. Solutions of erythromycin and spiramycin (500 μg./ml. of each) were prepared by dissolving 250 mg. of the pure substance in 0·5 ml. of ethanol and then diluting with sterile 0·85 per cent saline to the required concentration.

Stock antibiotic solutions were stored in the deep-freeze without loss of potency during the period of the investigation. For use stock solutions were diluted with 0·85 per cent saline on the day required.

Fibrin membranes.—Preliminary studies were made to determine the most satisfactory method of producing a standard type of fibrin membrane. The method finally adopted made use of a straight sterile sintered glass filter tube (porosity No. 1, internal diameter 10 mm., thickness of filter disc 3 mm.). The side arms were heated and bent to an angle of 45° midway between the filter disc and the ends of the tubes. A series of such tubes was prepared.

To make the fibrin membrane the filter tube was clamped vertically to a retort stand and the filter disc was saturated with fresh sterile human plasma from a donor. Immediately saturation was complete, usually with a volume of approximately 0·15 ml. of plasma, a drop of sterile commercial thrombin solution was added to cause clotting. The tubes were left for 30 min. and were then placed horizontally in the clamp. Simultaneously, 5·0 ml. of antibiotic test solution were added to the left-hand limb of the tube and 5·0 ml. of sterile saline (pH 7·0) to the right-hand limb. In this way unequal pressures on either side of the membrane were avoided and the volumes used were sufficient to cover both sides of the membrane completely.

At varying intervals of time 0·25 ml. volumes were removed from the right-hand limb and stored in the deep-freeze for subsequent assay of antibiotic content. A corresponding volume was also removed from the left-hand limb and discarded in order to equalize the pressures on either side of the membranes. A final check was also made on the potency of the antibiotic test solutions at the end of each period of observation.

Assay of test fluids. All assay procedures were carried out using serial doubling dilutions of the test fluid in digest broth in 0·5 ml. amounts. The test organism used for each was a stock culture of *Staphylococcus pyogenes* (var. *aureus*, N.C.T.C. 6571). The inoculum consisted of an 0·02 ml. amount of a 8-hr. digest broth culture. Results were read after 24 and 48 hr. of incubation.

Testing of fibrin membranes.—Intact fibrin membranes will retain the dye Congo red (Hughes, 1948), and this was made use of in determining the intactness of the membranes. A 0·1 per cent solution of Congo red was substituted for the fluid in the right-hand limb of the tubes, after the test period was over, and left for 6 hr. Any diffusion of the dye during this period was taken to indicate a possible defect in the membrane. With a little practice it was found that intact membranes could be produced without much difficulty.

RESULTS

The assay results obtained with the various antibiotics are detailed in the Table and represent the average findings of repeated observations made at varying intervals of time. Using Congo red as an indicator of the state of the membrane it was found that after about 60 hr. there was passage of the dye. After 48 hr. the membranes were intact but it was considered that observations beyond this period might be unreliable.

TABLE.—*Antibiotic Concentrations on the Opposite Side of a Fibrin Barrier at Different Time Intervals*

Time (hr.)	Antibiotic				
	Penicillin 2000 units/ml. (units/ml.)	Chlor-amphenicol 200 μg./ml. (μg./ml.)	Chlor-tetracycline 200 μg./ml. (μg./ml.)	Erythro-mycin 100 μg./ml. (μg./ml.)	Spiramycin 100 μg./ml. (μg./ml.)
1	300	0	0	0	0
2	400	0	2	0	0
3	800	1	4	0	0
4	900	2	5	0	0
5	1000	5	8	1	0
8	1000	8	10	1·5	0·5
10	1000	8	12	2·0	1·0
12	1000	10	18	3·0	1·5
16	1000	12	26	4·0	2·0
20	1000	16	28	4·0	2·5
24	1000	16	30	5·0	2·5
36	1000	18	35	6·5	3·0
48	1000	20	40	7·0	3·5
Theoretical final concentration assuming equilibrium	1000	100	100	50	50

The diffusibility of penicillin through this type of membrane was far superior to that of the other antibiotics tested, complete equilibrium with the test solution being attained in about 5 hr. A series of observations with penicillin concentrations ranging from 100 to 1,000 units/ml. showed no difference in the time taken for equilibrium to be reached. These observations are in agreement with the *in vivo*

findings of Weinstein *et al.* (1951) who showed rapid diffusion of penicillin into tissue-implanted clots in rabbits.

With erythromycin and spiramycin the maximum concentrations attained on the opposite side of the fibrin membranes were approximately 1/7 and 1/14 of the theoretical final concentrations assuming free diffusion across the membranes with equilibrium. Both chloramphenicol and chlortetracycline appeared to diffuse more freely but again the final concentrations after 48 hr. represent only 1/5 and 2/5 respectively of the theoretical equilibrium concentrations. Experiments carried out with oxytetracycline and tetracycline yielded results similar to those given by chlortetracycline.

Observations were made on the diffusion of penicillin, chloramphenicol and chlortetracycline at 4·0° and at 37·0°. With chloramphenicol and chlortetracycline there was slightly more rapid diffusion at 37·0° during the first twelve hr. than there was at room temperature but the final concentrations after 48 hr. were practically identical with those at room temperature. The rates of diffusion at room temperature and at 4·0° appeared to be similar. For penicillin there was no apparent difference in the rate of diffusion at all temperatures.

DISCUSSION

The rôle of fibrin barriers in inflammatory foci has received attention mainly from the point of view of the efficiency of the barrier in preventing organisms and toxins from passing into the general circulation. According to Menkin (1940), fibrin deposited in lymphatic channels and tissue spaces constitutes an important barrier to such passage. Hughes (1948) and Dubos (1955) have pointed out that fibrin acts in a selective manner allowing the passage of certain toxins but retaining micro-organisms although allowing the passage of leucocytes. Day (1954) found that the perfusion of mouse connective tissue with plasma in which clotting was in actual process led to a decrease in permeability.

Whether or not an antibiotic will pass through a tissue fibrin barrier is of considerable importance since complete eradication of an inflammatory focus may be largely dependent on adequate access of the drug to the site of infection. The present results show that under *in vitro* conditions fibrin barriers may exert a selective action on the passage of certain antibiotics. The choice of a suitable antibiotic may thus depend on the nature of the lesion in addition to the activity of the drug on the causative organism.

The diffusion of an antibiotic through fibrin is almost certainly a complex matter involving factors other than the pore size of the membrane and the concentration of the antibiotic. Some of the *in vivo* factors which may influence such diffusion are difficult of reproduction in an *in vitro* study. The constant biochemical changes taking place locally in the tissues which are the seat of infection may considerably alter the rate and amount of diffusion. In addition the pore size of the membrane both *in vitro* and *in vivo* will tend to alter as the result of the spiral configuration which the fibrin molecules tend to assume during the process of clot retraction. Amongst the other factors involved, diffusion may be influenced by chemical changes taking place between the antibiotic and the amino or carboxyl groups of the fibrin molecules. In addition the degree of binding of the antibiotic to the protein of the tissue fluids may affect the amount of diffusion. With regard to the local biochemical environment of the lesion alterations in the pH of the

fluid within the area of inflammation may be of importance. Dubos (1955), for example, has shown that in tissues which are the site of a staphylococcal infection there may be a considerable rise in pH associated with increased glycolytic activity and a fall in the tissue glucose content.

In the experimental model described here we have made use of a standard thickness of membrane but diffusion through an *in vivo* barrier will obviously be influenced by variations in the thickness of such membranes. In coagulase-producing staphylococcal infections very considerable amounts of fibrin may be deposited. Fibrinolysis, however, will also occur to a certain extent *in vivo*. Where streptococci produce large amounts of streptokinase the deposition of fibrin may be minimal. Part of the reason for the ultimate deterioration of the type of membrane made use of in this *in vitro* study is probably fibrinolysis due to the spontaneous conversion of plasminogen, adsorbed on the fibrin during the clotting process, to plasmin.

In the early stages of the formation of an inflammatory focus the increased tissue pressure around the lesion may be sufficient to allow the passage of an antibiotic which will not diffuse readily once the fibrin barrier is formed. With the formation of the inflammatory exudate the pressures inside and outside the lesion tend to reach equilibrium and the subsequent passage of antibiotic will then depend mainly on diffusion processes. Treatment, however, will seldom be instituted at the stage where the tissue pressures outside the lesion are above those in the lesion itself. Consequently we have ignored the effect of filtration pressures in these experiments in favour of the results of diffusion. It is not of course possible to draw any analogy between the arrangement of fibrin strands in tissue spaces and those in the interstices of a sintered glass filter since too many variable factors exist *in vivo* which cannot be allowed for in the experimental model. It is interesting, however, that Hughes (1948) found that the filtration of serum through fibrin membranes *in vitro* gave rise to a filtrate very similar in composition to that of an inflammatory exudate.

The findings for penicillin agree with those of Nathanson and Liebhold (1946) as well as those of Weinstein *et al.* (1951). The former workers also showed that penicillin would diffuse freely into agar. Masonyi, Held and Kocsan (1949), on the other hand, reported that penicillin would only penetrate fibrin layers greater than 3 mm. with difficulty. Werner, Knight and MacDermott (1954) also reported that penicillin, streptomycin, chloramphenicol and chlortetracycline diffused readily into agar discs *in vitro* and *in vivo*. It seems probable, however, that these findings have little reference to the problem of fibrin barriers in view of the known selective effect of the latter.

SUMMARY

The diffusion of five antibiotics through fibrin membranes has been investigated *in vitro*.

Penicillin diffuses rapidly through such a membrane attaining equilibrium on both sides of the barrier in five hours. The passage of chloramphenicol and chlortetracycline across such a membrane was considerably less, resulting in about one-fifth and two-fifths respectively of the theoretical equilibrium concentrations, after a period of 48 hours. Two other antibiotics, erythromycin and spiramycin, diffused across the membrane with still greater difficulty.

The relationship of these findings to *in vivo* conditions is uncertain since a number of factors are concerned which cannot be allowed for in the experimental model.

REFERENCES

DAY, T. D.—(1954) *Brit. J. exp. Path.*, **35**, 315.
DUBOS, R.—(1955) *Lancet*, ii, 1.
HUGHES, W.—(1948) *Brit. J. exp. Path.*, **29**, 173.
MASONYI, L., HELD, R. AND KOCSAN, C.—(1949) *Acta med. scand.*, **132**, 487.
MENKIN, V.—(1940) ' Dynamics of Inflammation,' New York (MacMillan).
NATHANSON, M. H. AND LIEBHOLD, R. A.—(1946) *Proc. Soc. exp. Biol., N.Y.*, **62**, 83.
WEINSTEIN, L., DAIKOS, G. K. AND PERRIN, T. S.—(1951) *J. Lab. clin. Med.*, **38**, 712.
WERNER, C. A., KNIGHT, V. AND MACDERMOTT, W.—(1954) *J. clin. Invest.*, **33**, 742.