HISTOLOGICAL EFFECTS OF SULPHONAMIDE-PROFLAVINE MIXTURES IN THE RABBIT

SOME EXPERIMENTAL OBSERVATIONS

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The widespread use of sulphonamide powders in the treatment of wounds has prompted the idea that other substances—notably the acridine antiseptics—might be applied in a similar manner. Thus Mitchell and Buttle (1942, 1943) have claimed beneficial effects from the use of proflavine and diflavine in war wounds. Experiments have shown, however, that acridine powders have a highly destructive effect upon the tissues (Russell and Falconer, 1943; Hawking, 1943). The use of these powders in undiluted form in the treatment of fresh wounds therefore seems undesirable, though they are not of necessity contraindicated for secondarily infected wounds which have failed to respond to other therapeutic measures.

A substantial dilution of proflavine with one of the sulphonamides might be considered a suitable mixture for the prophylactic treatment of wounds, and a blend of one part of proflavine powder in 100 or 200 parts of sulphathiazole has been suggested by McIntosh and Selbie (1943), who find that proflavine is more potent than the sulphonamides in infections with some of the gas-gangrene organisms. Sulphathiazole powder has been found by Glynn (1942) to have a negligible damaging effect upon the tissues in experimental wounds, and in this respect is preferable to either sulph-anilamide or sulphapyridine.

The present experiments demonstrate in rabbits the effects of proflavine-sulphonamide mixtures upon (a) muscle and subcutaneous tissues, and (b) the brain.

Muscle and Subcutaneous Tissues

In this group the powder tested was composed of 1 part of proflavine sulphate and 99 parts of sulphathiazole. The experiments were similar to those described by Glynn. Under ether anaesthesia an incision 1 to 2 cm. long was made in the depilated skin of the thigh, and a pocket (about 2.5 cm. diameter), made between the panniculus carnosus and the skeletal muscle by introducing and opening the blades of blunt scissors, was filled with about 0.25 g. of the powder. The wound was then closed. The disk of blood-clot also inserted by Glynn was omitted. Six rabbits were treated in this way, sulphathiazole alone being introduced into the left leg, and the mixed powder into the right. The animals were killed after 1, 2, 4, 8, 10, and 16 days respectively.

MACROSCOPICAL CHANGES

The site of operation in general appeared swollen and felt boggy for the first few days after operation. In four of the animals the right side was considerably more swollen than the left, and in one of these slight oozing of clear yellowish fluid was observed on the third day at the point where a stitch had been lost. From the fourth day onwards this wound was dry and the inflammation subsided. In the remaining two animals the left leg showed the greater swelling. All wounds appeared to heal in a normal manner with the single exception mentioned.

When the block of tissue including the operation area had been excised, fixed in formaldehyde, and sliced, the powder was identified as a lenticular, cheesy mass about 2 cm. in diameter and 0.3 or 0.4 cm. thick, which decreased with time and was reduced to a linear streak by the eighth day. With *pure sulphathiazole* the changes in the surrounding tissues were limited to slight oedema on the first day, with traces of haemorrhage in the adjacent fasciae and deep aspect of the skin. With the *mixed powder* there was on the first day severe oedema with diffuse haemolytic staining of the deep aspect of the skin, extending from the site of operation to the base of the tail and involving the fascia covering the skeletal muscle for a distance of 1 cm. from the pocket. On subsequent days the staining of the fascia was replaced by petechial haemorrhages, which similarly extended for distances up to 4 cm. from the site of opera-

tion. There was a zone of discoloration up to 2 mm. deep in the skeletal muscle, varying from yellow on the first day to brownish pink or yellowish grey in later stages. Oedema of the adjacent tissues, and particularly of the skin, persisted up to the sixteenth day over the pocket, which was replaced after the eighth day by a lenticular mass of gelatinous greyish-yellow material.

MICROSCOPICAL CHANGES

1. *With sulphathiazole alone* oedema of the dermis, panniculus carnosus, and the deep fascia was conspicuous at first, but had almost disappeared by the fourth day. Most of the powder had been absorbed by the fourth day, but a few crystals were found on the tenth. The site of the powder beneath the panniculus carnosus was identified by a capsule of closely felted filaments of fibrin, which became increasingly folded as the space collapsed. By the fourth day this capsule was sparsely infiltrated by polymorphonuclear leucocytes and large mononuclear cells, and immediately about it lay a thin zone of proliferating fibroblasts. By the eighth day the capsule was broken at many points by the ingrowth of granulation tissue, which lined most of the enclosed space (Fig. 1). The

FIG. 1.—Border of skeletal muscle and lateral angle of pocket 8 days after insertion of sulphathiazole powder. The borders of the pocket are replaced by granulation tissue. H. and E. $\times 21$.

appearances were similar on the tenth day, but by the sixteenth day all traces of the cavity had been obliterated by collagenous tissue. The panniculus carnosus immediately over the pocket was involved by the formation of granulation tissue, but the muscle fibres, though separated, did not undergo degeneration or necrosis. The subjacent skeletal muscle of the thigh showed slight oedema in early stages but underwent no degeneration in any instance.

2. *With sulphathiazole-proflavine mixture* the histological changes differed from those which followed the introduction of sulphathiazole alone, first, in the persistence of local oedema up to the sixteenth day; secondly, in the conspicuous development of haemorrhagic necrosis in the dermis, the panniculus carnosus, the subjacent skeletal muscle and its fascia (Fig. 2); and, thirdly, in the consequent retardation of healing in these layers although the skin incision healed as rapidly as in the controls. Thus the panniculus carnosus was completely necrotic throughout the length of the section (2.5 cm.) in the animal killed after one day, and the necrosis extended laterally for a similar distance in the subjacent fascia, which was greatly swollen. Yet the zone of necrosis in the skeletal muscle was no deeper than about 1 mm., and in two of the animals was limited to the most superficial fibres. The extent of necrosis about the pocket of powder resulted in a retardation of healing. Thus granulation tissue did not appear until the eighth day; it then occupied the deep border of the subjacent necrosed skeletal muscle and the surface of the deep fascia over it lateral to the pocket, while tongues of young fibroblasts penetrated through the subcutaneous tissue to within 2 mm. of the fibrin capsule about the pocket. Even by the sixteenth day the fibrin capsule persisted at many points, especially along the superficial border towards the dermis.

COMMENT

The histological effects of sulphathiazole powder alone are thus negligible, confirming the observations already made by Glynn. A mixture of proflavine sulphate and sulphathiazole (1:100) is substantially damaging to muscle and connective tissue: necrosis and haemorrhage are widely distributed along fascial planes and in loose connective tissue, but the penetration of solid masses of muscle appears very limited.

Since these observations were made some parallel experiments on rats have been reported by Selbie and McIntosh (1943). They inserted about 10 mg. of similar powders beneath the panniculus carnosus of the abdominal wall on either side of the mid-line. The area exposed to the powders was of similar dimensions to that in our experiments. The histological results in these rats appear to have resembled those obtained by us in the rabbit except in two particulars. First, the

FIG. 2.—Same animal as in Fig. 1, showing necrosis of skeletal muscle and fascia over it 8 days after insertion of proflavine-sulphathiazole mixture. H. and E. $\times 21$.

panniculus carnosus in the rat underwent the same degree of necrosis following exposure to both kinds of powder. Secondly, no retardation of healing was observed in the rat in comparison with the areas treated by sulphathiazole alone, in spite of the apparently greater damage inflicted by the proflavine mixture. To some extent these divergences might be attributable to the use of different animals and different sites. But more important, probably, is the difference in the severity of the test. Thus in the experiments of Selbie and McIntosh a much smaller quantity of the powder was dispersed in a thin film between the muscle layers, while we, following Glynn, inserted enough powder to form a lenticular mass several millimetres thick. The observed difference in the two series is a warning against the use of excess of the proflavine-sulphathiazole mixture.

Central Nervous System

Sulphapyridine was substituted for sulphathiazole in these experiments on account of the known epileptogenic effects of the latter. In a few animals sulphadiazine was used. Craniotomy was performed on 28 rabbits, the technique being the same as in earlier experiments (Russell and Falconer, 1943). Details are given in the Table.

The animals were killed at either 2 or 4 days after the operation. During this period weakness of the right limbs and excitability were noticed in both animals in Group 1, and to a less marked extent in a variable number of animals in all the other groups.

Pathological examination showed a lack of uniformity in the reaction of the meninges and brain in the exposed area. In Groups 1 to 3 inclusive a proportion of the animals, as shown in the Table, exhibited a haemorrhagic necrosis that

Group	Mixture Used	No. of Animals	Severe Reaction
1 ..	Proflavine sulphate - sulphapyridine,	1:25 ..	2 1
2 ..	" ..	1:50 ..	7 3
3 ..	" ..	1:100 ..	9 3
4 ..	" ..	1:200 ..	7 0
5 ..	" .. - sulphadiazine,	1:200 ..	3 1

extended down to the wall of the lateral ventricle. In the rest the reaction was limited to focal meningeal haemorrhage and oedema of the cortex, accompanied by severe degeneration, with or without necrosis, of a superficial zone not exceeding 1 mm. in depth. In Group 4 this zone did not exceed 0.5 mm. in depth, and the reactions in this group were thus of a more consistent character though somewhat variable.

No satisfactory explanation of these uneven results can be offered. Examination of the powder under low powers of the microscope suggested that uneven distribution of the proflavine particles might be responsible, but regrounding the mixture in an agate mortar, and trials of specially prepared samples kindly provided by Messrs. May and Baker Ltd., made no obvious difference to the results. Nor did accidental trauma to the brain during the operation appear to be a factor. Thus superficial trauma, due to a bleeding vessel or to a small abrasion, was noted in five of the animals. In two of these the ultimate damage to the brain was found to be severe: in the other three it was slight. Small variations in the amount of powder used might be regarded as a possible cause, but in one of the animals in Group 3 an excess of powder was deliberately applied, and in this animal the damage was limited to marginal oedema, haemorrhage, and necrosis, together less than 1 mm. deep. We are thus left with the unsatisfactory suggestion that variations in the results are due to factors inherent in the individual. It is nevertheless clear, as indicated in the Table, that the incidence of a severe reaction falls with a diminishing proportion of the proflavine used in the mixture.

Conclusions

The first group of experiments here reported confirms previous observations that the insertion of substantial amounts of sulphathiazole powder into clean incised wounds is harmless to the tissues. Similar amounts of a powder consisting of 1 part of proflavine sulphate to 100 parts of sulphathiazole caused considerable damage to the neighbouring muscle and connective tissues, though the penetration of the proflavine into solid masses of muscle appeared to be slight and rather uneven.

The experiments on the rabbit's brain with mixtures of proflavine and sulphapyridine powders indicate a similar damaging effect of the proflavine, though the results in this group are not so clear-cut. Mixtures containing only 0.5% of proflavine sulphate are appreciably less damaging than those with higher percentages of this compound. Comparison with earlier experiments shows that both proflavine solutions (1:1000) and sulphonamide powders are preferable from the standpoint of histological effect.

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REFERENCES

- Glynn, L. E. (1942). *J. Path. Bact.*, **53**, 183.
Hawking, F. (1943). *Lancet*, **1**, 710.
McIntosh, J., and Selbie, F. R. (1943). *Ibid.*, **1**, 793.
Mitchell, G. A. G., and Buttle, G. A. H. (1942). *Ibid.*, **2**, 416.
— (1943). *Ibid.*, **2**, 287.
Russell, D. S., and Falconer, M. A. (1943). *Ibid.*, **1**, 580.
Selbie, F. R., and McIntosh, J. (1943). *J. Path. Bact.*, **55**, 477.

The National Institute for the Blind in its 74th annual report records a year of remarkable activity among the hundreds of sightless physiotherapists trained at the Institute's School of Massage and now engaged in the work professionally. At the clinics attached to the London headquarters there have never been so many patients in attendance or so many treatments given. The numbers at the evening clinic were respectively 7,610 and 14,117 as compared with 5,185 and 9,859 in the previous year; and at the Eichholz Clinic, which is now recognized as a fracture clinic by the Ministry of Health, they were 4,800 and 11,616, as against 2,972 and 7,657.