BRITISH MEDICAL JOURNAL

LONDON SATURDAY MARCH 20 1943

THE PRESENT STATUS OF AMINOACRIDINE COMPOUNDS (FLAVINES) AS SURFACE ANTISEPTICS

BY

C. H. BROWNING, M.D., F.R.S.

(From the Bacteriology and Pathology Department of the University and Western Infirmary, Glasgow)

Infected war wounds again demand efficient chemotherapy as an adjunct to surgery; and while fulfilment of the high promise of penicillin waits on ample supplies, limitations in the performance of the sulphonamide series present themselves (Spooner, 1941; Francis, 1942; Mitchell and Buttle, 1942). The last-named workers have now returned to proflavine. Acriflavine and its precursor, proflavine (salt of 2:8-diaminoacridine), were introduced by us in 1917 as surface antiseptics because—they are powerfully bacteriostatic towards the important pyogenic organisms; serum does not diminish this action, although blood somewhat reduces it and pus greatly; toxicity for the body as a whole is not high; they are comparatively unirritating; and phagocytosis is little interfered with. It is appropriate to review briefly the present status of the aminoacridine group of drugs ("flavines") and possibilities of future improvements in them. Experimental results will be stressed, since patients should be subjected to new compounds only after their action has been closely studied in the laboratory. Further, experiments can be duplicated with the minimum of variation, which is difficult clinically.* When drugs with outstanding properties are discovered in the laboratory it remains for clinical skill to utilize them to full advantage.

Antiseptic Action

The diaminoacridine compounds proflavine and acriflavine are antiseptics whose action is intensified rather than reduced when the medium contains serum (Browning and Gilmour, 1913). This feature distinguishes them from all the powerful older antiseptics. When surgical experience after 1914 clamoured for improved treatment, these antiseptics were studied anew and proved highly potent. In serum, concentrations of 1:100,000 to 1:200,000 sterilize *Staphylococcus aureus* and typical *B. coli*, and in dilute peptone water 1:200,000 and 1:20,000 respectively. *Streptococcus pyogenes* is even more susceptible. Such high values are attained slowly, after 24 hours' contact—bacteriostatic action. However, brief contact with the drug, short of producing death, reduces the virulence of the organisms. *B. pyocyaneus*, *B. proteus*, and certain atypical *B. coli* may be highly resistant. Enhancement of antiseptic action is not displayed in a medium with only a low concentration of serum (10%). Although action is increased at a slightly alkaline *p*H, this may not entirely explain the serum effect. In defibrinated blood the killing rate is slowed (Rubbo *et al.*, 1942); nevertheless there is powerful antisepsis. Pus greatly reduces antiseptic action, although not invariably (Gay and Morrison, 1921).

Acriflavine of commerce usually contains some of its precursor, proflavine; but mixtures of the two in varying proportions differ little in antiseptic action (Berry, 1941). These dyes are strongly fixed by tissues and cotton fabrics, etc.: thus after 3 minutes' soaking in 100 c.cm. of acriflavine 1:1000 the fluid expressed from an average 5-g. gauze swab contains 1:1600 of the antiseptic; with 1:5000 the expressed fluid contains 1:9000 (Graham). In applying wet dressings allowance must be made for this.

Recently Albert *et al.* (1938) and Rubbo *et al.* (1942) have introduced potent new acridine antiseptics, especially 2:7-diaminoacridine and 5-aminoacridine, the latter having the great advantage of being non-staining.

Action on Cells and Tissues

Toxicity for the Animal as a Whole.—Toxicity of a drug often varies remarkably according to the mode of application; a striking illustration is the poisonous action of iron parenterally, as compared with its innocuousness in the alimentary tract. Amounts of antiseptics likely to be absorbed from wounds should not be harmful to the body as a whole. On subcutaneous injection into mice the "average lethal dose" of proflavine which causes the death of 50% of the animals in 2 to 3 days is 0.2 g. per kg. of body weight; of acriflavine, 0.05 g. (Albert *et al.*, 1937). Accordingly, even the latter is not "a substance of great toxicity to animal tissues." In the treatment of gonorrhoea intravenous doses of 0.1 g. thrice weekly (Jausion *et al.*, 1931)† or 10 doses each of 0.04 to 0.08 g. at 2- or 3-day intervals (Assinder) have been well tolerated. These amounts are equivalent to 40 to 100 c.cm. of 1:1000 solution; thus toxic damage in man from acriflavine or proflavine absorbed from wounds has not occurred. Skin idiosyncrasy has been met with, although very rarely (Young and Hawking). 2:7-diaminoacridine is less toxic for mice than proflavine, and 5-aminoacridine is intermediate between proflavine and acriflavine. At the site of injection of flavines into the *closed* tissues necrosis tends to occur with the higher concentrations, hence care should be exercised in this method of administration.

Epithelium.—Irritation of the rabbit's conjunctiva is very slight after 3 minutes' contact with 1:50 proflavine solution or 1:150 acriflavine; in contrast, brilliant green 1:1000 is irritating, which corresponds with its effect on the human urethral mucosa. By this test the suitability of drugs for the local treatment of gonorrhoea was estimated. However, since irritation of wound surfaces is not caused by brilliant green, such results again emphasize the importance of the mode of application.

Brain.—Russell and Falconer (1941) exposed part of a cerebral hemisphere in rabbits and applied to the surface for 10 minutes "lintine" soaked in a solution of antiseptic; simultaneously 0.1 c.cm. was injected into the cortex. 1:1000 solutions of proflavine and 2:7-diaminoacridine in isotonic saline buffered at a *p*H of 6.2 were practically harmless, like neutral isotonic saline, judging by histological examinations made 1 to 8 days later. On the other hand, acriflavine 1:1000 and euflavine 1:2000, like most other antiseptics, caused marked haemorrhage and necrosis. The effect *in vitro* of antiseptics on the respiration of brain tissue paralleled the above

* For a thorough discussion of the principles of investigating chemotherapeutic antiseptics, see Garrod, L. P., *Lancet*, 1940, **1**, 798, 845.

† 97,000 injections were given to about 6,000 patients. In some cases the dose was 0.2 or 0.3 g.

results (Manifold, 1941). The tolerance to compounds of proflavine type is clearly of high significance for brain surgery.

Leucocytes.—In the usual opsonic experiments *in vitro* the presence of 1 : 500 to 1 : 1000 acriflavine or proflavine reduced the phagocytosis of a suspension of pyogenic staphylococci or streptococci to about 50% of the control. 5-aminoacridine behaved similarly, while the 2 : 7 derivative was somewhat more inhibitory (Albert *et al.*, 1938; Rubbo *et al.*, 1942). With mercuric chloride, iodine, or crystal violet 1 : 3500 to 1 : 7000 led to a similar reduction. After 2 hours' contact with 1 : 10,000 of the acridine drugs the phagocytic power of the leucocytes was little affected; according to Fleming longer contact with higher dilutions caused damage. The tests of Welch *et al.* (1942) indicated that acriflavine is not highly toxic for leucocytes. Albert *et al.* (1938) found that polymorphonuclear leucocytes after contact with 1 : 2000 proflavine or acriflavine for 90 minutes at 38° C. were still moderately motile, although stained bright yellow. But Abraham *et al.* (1941) recorded rapid high toxicity with proflavine. Bond (1917) had shown previously that, as measured by the iodophil reaction, leucocytes in granulation tissue were not seriously injured by contact with preparations containing 1 : 1000 acriflavine. Fleming concluded that even a momentary application of flavine 1 : 1000 to the leucocytes sufficed to destroy their bactericidal power. However, Gay and Morrison's observations on mixtures of acriflavine, virulent streptococci, specific antiserum (heated), and leucocytes showed that (*a*) low concentrations of the drug (1 : 128,000) produced sterilization, and (*b*) in its absence sterilization did not occur even when fresh normal serum (alexin) was added also. These extreme divergencies suggest that the workers' methods decide their results *in vitro*.

Tissue Culture.—According to Mueller, Hata, Wolff, and others, proflavine, acriflavine, and certain related compounds are distinguished by inhibiting streptococci in concentrations which are the same as or less than those damaging the tissues. On the other hand, Jacoby, Medawar, and Willmer (1941) found proflavine highly toxic for fibroblasts, macrophages, etc. Here again gross discrepancies appear. Since "culture" conditions represent a *tour de force* for animal cells rather than a normal state, comparisons reflecting adversely on the behaviour of cells in contrast to bacteria probably have little applicability *in vivo*. There are pathologists expert in tissue culture technique who consider that meanwhile this is too deeply involved in difficult problems of its own to be able to offer at the best more than a partial contribution to the solution of others.

Granulation Tissue.—I am indebted to Prof. D. F. Cappell and Dr. H. E. Hutchison (Pathology Department, St. Andrews University) for unpublished observations on absorption from treated wound surfaces. Flask-shaped wounds were made in the skin and subcutaneous tissues of rats by a special method; into these were introduced gauze and the solutions to be tested. Following the application of 1:1000 proflavine or acriflavine several times daily, the wound surface failed to develop impermeability to tetanus toxin, which occurs in untreated wounds usually within a week. Similarly, granulating wounds which had acquired impermeability became permeable again after treatment for some days. There was no difference in action between the two drugs under these conditions. On histological examination scantiness or alteration of the granulation tissue lining was a feature of the permeable wounds. But generalizations regarding permeability to toxins are not possible, because it was found that while newly inflicted wounds were permeable to the toxin of *Cl. oedematiens*, a state of impermeability set in after 18 hours; and this condition was not affected by the antiseptics. Further, in such experiments the anti-infective property of the drugs was intentionally excluded by using preformed toxins. Actually, these treated wounds were kept practically free from pyogenic infection, which occurred conspicuously in the untreated controls.

Chemotherapy of Recent Infections

Inoculation of recent wounds in guinea-pigs with virulent *B. diphtheriae* or in mice with virulent streptococci, or intraperitoneal inoculation of the latter, affords a most valuable test object. Failure to devitalize the organisms in each case quickly leads to death of the hosts. With *B. diphtheriae* brief washing of the infected wound with acriflavine solution (1 : 100 to 1 : 2500) 1 to 2 hours after inoculation regularly saved the animal's life. Washing with isotonic or 5% saline failed (13 acute deaths out of 14 animals), as usually also did treatment with carbolic acid 1:20 to 1:100 or bipp. Successful results with aminoacridine compounds have been repeatedly obtained in recent wounds of mice inoculated with streptococci. So, too, after intraperitoneal inoculation one intraperitoneal injection of the antiseptic after an hour's interval saved the life of 60% (Browning *et al.*, 1931). It is noteworthy that drug treatment of wounds infected with streptococci may succeed although excision fails to prevent general infection. An important fact is that a dose of the antiseptic sufficient to save the animal's life may, in the same concentration, take some hours to kill the streptococci *in vitro*. Thus the therapeutic action is clearly a co-operative effect—the defensive powers of the host disposing finally of organisms whose virulence has been reduced first by the drug. This reduction in virulence would explain clinically successful suture of treated wounds whose surfaces still harbour many organisms. These drugs also are effective in some infections with anaerobes. Recently McIntosh and Selbie (1942) inoculated mice intramuscularly with at least 100 times the fatal dose of *Cl. welchii* and then treated the site by injecting a drug. A well-tolerated dose of proflavine (0.5 mg.) was somewhat more efficacious in preventing death than 80 times as much sulphanilamide or sulphathiazole. Both classes of compounds acted when injected after an interval sufficient for some penetration of the organisms into the tissues, whereas zinc peroxide failed under the latter conditions. Hawking (1941) likewise found proflavine effective in guinea-pigs. Accordingly, *so far as the aminoacridine antiseptics are concerned, an unequivocal "yes" can be given to the question, "Is it possible, by the use of an antiseptic, to destroy an infection in a freshly inflicted wound before the bacteria have had time to grow out?"*

There is no evidence that subcutaneous and muscular tissues of animals behave so differently from those of the human that what holds true in principle of the former will fail in the latter. Successful prophylactic treatment of recent wounds in man with acriflavine or proflavine has often been recorded (Drummond and McNee; Colledge *et al.*, 1917). Two criticisms have been expressed. First, the results could be obtained equally by débridement alone. In view of the experimental work this objection falls. Secondly, "Is it justifiable for the surgical pessimist who distrusts his own cleanliness to use antiseptics in an attempt to cover up his own deficiencies?" This taunt ignores the fact that even surgical wounds are sites of diminished resistance and so are liable to infection—e.g., from the circulation. The development of spontaneous metastatic gas gangrene affords clear evidence of this route of infection (Learmonth, 1924). *Suitable antiseptics should be used prophylactically for all wounds*, simply because no one can foretell when, without their aid, even a minor trauma will result in major sepsis. Charteris's work with the oily preparation of proflavine oleate for avoidance of sepsis in radium therapy is another outstanding surgical application.

Treatment of Established Local Sepsis

Suppurating wounds as met with in man are not readily imitated in laboratory animals. Consequently one must examine well-controlled clinical evidence. For example, in Carslaw's case (No. 15), suffering from extensive bomb wounds of the face, great loss of tissue, and much suppuration in spite of treatment successively with iodoform gauze and moist eusol dressing, resort to flavine was followed in two days by great improvement in the general state, the inflammatory condition of the skin and soft tissues rapidly diminishing and large sloughs separating. "This case illustrates the rapidity with which flavine can clean up a foul lacerated wound which was not yielding to other methods of treatment." Graham recorded an unexceptionable experiment: three fairly severe burns of similar size affecting the hand and forearm—the whole depth of the epithelium being destroyed—were treated within an hour with acriflavine. After two days the dressing on the least severe burn was changed to wet boric acid lint, whereupon, unlike the others, it became painful and suppurated; return to acriflavine restored the good progress.

Carslaw's general method in war wounds was to secure an "open" wound with adequate drainage, any accessible foreign body being removed. Strips of gauze well soaked in 1:1000 flavine were lightly packed into the depths of the wound, which was then covered with folds of gauze similarly soaked, the whole being covered with waterproof. The folds of gauze were changed once or twice a day, but the packing was not as a rule removed until 48 hours after introduction. Bashford

and his co-workers, while emphasizing defects of treatment with these antiseptics, nevertheless stated that "pus was generally very small in amount, and even where it had been present it usually rapidly diminished in quantity. . . . Any spreading infection that already existed generally subsided quickly. . . . The patient is apparently protected in some way from the absorption of toxic products"—surely no mean achievement. Recently in the Middle East Mitchell and Buttle have dealt with "wounded men who, either in spite of chemotherapy or more probably owing to its inadequate application, arrive at the base with their wounds in a grossly infected and purulent condition. In these cases the sulphanilamide drugs can do no more than prevent the spread of infection and they have comparatively little influence on the course of the local suppuration." The wounds were commonly deep or extensive, often involving bones or joints. The novel procedure of applying proflavine in dry powder form was adopted, it being spread over the whole surface of extensive wounds or cavities—never exceeding 2 g. in any case, and usually only about 0.5 g. or less, depending on the size of the lesion. It was never employed on more than two or three consecutive occasions, at intervals of 4 to 28 days. Most patients found the dressing painless. Improvement followed proflavine irrespective of the type of infective organism; and good results might be attained in cases which had failed to respond to one of the sulphonamide series. No other drug was as effective as proflavine in controlling or eliminating staphylococcal infection. Oily preparations of proflavine oleate, originally used by Berkeley and Bonney for dressing large raw surfaces, are also valuable—e.g., on septic sites preparatory to radium therapy (Charteris, 1937).

Influence on Healing

After 4 to 7 days' treatment a yellowish fibrinous membrane often covered the wounds (Carslaw; Drummond and McNee; Colledge *et al.*). At this stage the flavines had fulfilled their therapeutic purpose in preventing or overcoming sepsis, and a change of treatment was indicated—e.g., on using eusol the pellicle disappeared, while granulation and growth of epithelium proceeded (Carslaw). Bashford and his collaborators, working at the Surgical Observation Hut organized by Surg. Gen. Sir George Makins, picked for trial with the flavines 50 patients of whom they said "almost all had been treated previously at the front by the Carrel-Dakin method within 24 hours of being wounded, and, in the majority of instances, very efficiently." The reason for this selection of cases already effectively treated by another method is hard to explain. However, despite the beneficial effects mentioned above, they chiefly dwelt on the delay in all the processes of repair; later, this work was quoted extensively by Makins (1922) in the *Official History of the War*. Pilcher and Hull, writing at the time from their own experience of over 5,000 cases in military hospitals, made the following comment: "For ease of preparation and application, rapidity when dealing with large numbers of cases, complete absence of surgical fidget, early cleaning of the wounds, and abatement of constitutional reaction to absorption, flavine is an admirable application under all circumstances, but especially where surgeons are few, time is short, and wounds are many." The factors in treatment which may retard healing have not yet been fully defined. Bennett did not experience it in over 600 cases. In tissues from this series Blacklock found mitoses of various cell types at levels of 1/50 to 1/5 mm. below the actual growing surface. The margin between the optimum and too high a concentration of the drug due to frequent renewal or evaporation may be narrow. A modified treatment consisting of less frequent applications and weaker solutions—1 : 5000 or under—in later stages has not been widely enough tried. Mitchell and Buttle attributed possible interference with healing to proflavine powder only once in 80 cases.

Conclusions

1. Aminoacridine ("flavine") compounds were introduced as surface chemotherapeutic agents for wounds because they combined outstanding antiseptic properties with relatively low toxicity, as demonstrated by simple methods *in vivo* and *in vitro*. Later tests *in vitro* have yielded discordant results *inter se*, which throw doubt on their value as a practical means of selecting drugs for such purposes.

2. Proflavine is the precursor of acriflavine, and so cheaper. Also, while equally antiseptic, proflavine is the less toxic generally, and especially is very little harmful to brain tissue. Recently introduced members of the series may prove even more suitable for therapy.

3. It has been shown under experimental conditions that the flavines when applied to the tissues at the site of inoculation are highly effective in preventing the development of infection with various organisms, including streptococci and certain gas-gangrene anaerobes.

4. Also, there is good clinical evidence that they can control established suppuration in wounds. This may occur where drugs of the sulphonamide group have failed.

5. Retardation of granulation and healing tends to follow continued use of the flavines, so that it may be advantageous to resort to other treatment later. But the point important practically is the capacity of the flavines to prevent or control infection. This so-called "pickling" or "cold-storage" effect has enabled wounded men to be transported without re-dressing, in the fair likelihood that their wounds would remain *in statu quo ante* as regards infection.

Bibliography

[Earlier references omitted here will be found in: Bennett, C., Blacklock, J. W. S., and Browning, C. H. (1922). *British Medical Journal*, **2**, 306; Browning, C. H. (1937). *Edinb. med. J.*, n.s. (4th), **44**, 497 (Cameron Prize Lecture for 1936); and Browning, C. H. (1940). *Practitioner*, **145**, 293 (contains a full account of clinical uses).]

Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N. G., Jennings, M. A., and Florey, H. W. (1941). *Lancet*, **2**, 177.
Albert, A., Dyer, F. J., and Linnell, W. H. (1937). *Quart. J. Pharm. & Pharmacol.*, **10**, 649.
—— Francis, A. E., Garrod, L. P., and Linnell, W. H. (1938). *Brit. J. exp. Path.*, **19**, 41.
Berry, H. (1941). *Quart. J. Pharm. & Pharmacol.*, **14**, 149.
Bond, C. J. (1917). *British Medical Journal*, **2**, 6.
Charteris, A. A. (1937). *Lancet*, **2**, 627. (See also Young and Charteris.)
Colledge, L., Drummond, H., Worthington, R. T., McNee, J. W., Sladden, A. F., and McCartney, J. E. (1917). Ibid., **2**, 676.
Fleming, A. (1940). *Proc. roy. Soc. Med.*, **33**, 487.
Francis, A. E. (1942). *Lancet*, **1**, 408.
Gay, F. P., and Morrison, L. F. (1921). *J. infect. Dis.*, **28**, 1.
Hawking, F. (1941). *British Medical Journal*, **1**, 263.
Jacoby, F., Medawar, P. B., and Willmer, E. N. (1941). Ibid., **2**, 149.
Jausion, H., Pecker, A., and Medioni, G. (1931). *Soc. méd. Hôp. Paris*, 3 sér., **47**, 397—see *Presse méd.*, **39**, 358.
Learmonth, J. R. (1924). *Lancet*, **2**, 648.
McIntosh, J., and Selbie, F. R. (1942). Ibid., **2**, 750.
Makins, G. H. (1922). *Official History of the War: Medical Services: Surgery*, **1**, 248, London.
Manifold, M. C. (1941). *Brit. J. exp. Path.*, **22**, 111. (Contains a review of the general toxicology of acriflavine.)
Mitchell, G. A. G., and Buttle, G. A. H. (1942). *Lancet*, **2**, 416.
Mueller, J. H. (1918–19). *J. Path. Bact.*, **22**, 308.
Rubbo, S. D., Albert, A., and Maxwell, M. (1942). *Brit. J. exp. Path.*, **23**, 69.
Russell, D. S., and Falconer, M. A. (1941). *Brit. J. Surg.*, **28**, 472.
Spooner, E. T. C. (1941). *J. Hyg., Camb.*, **41**, 320.
Welch, H., Slocum, G. G., and Hunter, A. C. (1942). *J. lab. clin. Med.*, **27**, 1432.
Wolff, L. K., and Julius, H. W. (1939). *Ann. Inst. Pasteur.*, **62**, 616.
Young, G., and Charteris, A. A. (1940). *British Medical Journal*, **2**, 489.

HERNIA THROUGH THE FORAMEN OF WINSLOW, EMERGING THROUGH THE GASTRO-HEPATIC OMENTUM

BY

Brigadier HAROLD EDWARDS, M.S., F.R.C.S.
Consulting Surgeon to Southern Command

AND

Major W. STEWART, M.D.
Pathologist, R.A.M.C.

Hernia through the foramen of Winslow into the lesser sac is one of the more uncommon abdominal surgical conditions, and the re-entry of the herniated bowel back into the general abdominal cavity through the lesser omentum appears to be still more rare. The transverse colon probably acts as a barrier to herniation. The anterior and posterior boundaries of the foramen during life are in direct contact, and the opening is a slit-like passage (Cunningham's *Anatomy*, 7th ed., p. 576).

Moynihan and Dobson (1906) state that herniation through this foramen requires one of the following conditions: (*a*) a common mesentery of the whole intestine; (*b*) absence of