THE ANTIBACTERIAL ACTION OF COLCHICINE AND COLCHICEINE

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COLCHICINE is found in various proportions in all parts of *Colchicum autumnale* and other colchicum species, being most abundant in the ripe seed, especially the seed-coat. It occurs as a white amorphous mass, readily soluble in water, in ethanol and in chloroform.

Colchiceine is a decomposition product of colchicine which may be prepared by heating it with dilute sulphuric or hydrochloric acid. According to Oberlin[1], colchiceine is found with colchicine in the plant; but according to Zeisel[2], it is formed during the extraction of colchicine. The latter is believed to be a methyl ether of the former. It occurs in colourless glistening needles, readily soluble in alcohol, in chloroform, and, sparingly, in water.

Colchicine is a mitotic poison which produces cellular changes similar to those produced by X rays, and is probably the most powerful "radiomimetic" drug known. In very high dilutions it is said to bring about a typical mitotic arrest at the metaphase stage. Amoroso[3] carried out a series of experiments with the object of determining to what extent colchicine might affect new growth. The results showed that this alkaloid can bring about regression and final disappearance of tumours in mice.

Ludford[4] examined the mode of action of colchicine on tissues grown *in vitro*, and observed that the drug did not differentiate between embryonic and neoplastic tissues. Both were equally sensitive to its mitosis-arresting activity. Other chemical and physical agents may also inhibit mitosis, but according to this author, "colchicine is unique in that it does not arrest the initial phase of division but brings the process to a standstill at the metaphase in a remarkably wide range of concentrations." Macroscopically it was observed that the drug produced hæmorrhage similar to that produced by the polysaccharide fraction of certain bacteria, e.g., *Chromobacterium prodigiosum*. Both of them are believed to attack the sensitive cells of the capillary system of a rapidly growing tumour. The same author observed also that mitosis could be arrested with a very small amount of colchicine, far smaller than that capable of producing metabolic disturbance in the cell. Indeed, dark ground examination of cells under the influence of colchicine revealed little alteration in the mitochondria and in the cytoplasmic granulation, indicating that the metabolic mechanism was not affected. In one experiment it was found that the dose required to produce a metabolic effect was a thousand times bigger than that required to arrest mitosis. The same author[5] made a detailed investigation of the factors determining the action of colchicine on tumour growth, using a rapidly growing

carcino-sarcoma of the Strong A strain of mice. After repeated administration of the drug he noticed a sudden decline in the size of the tumour, but some days after cessation of treatment growth was resumed: and he came to the following conclusions: (1) colchicine produces regression of tumours which is often followed by recurrence; (2) it is more active on rapidly growing than on slow-growing tumours, the reaction of the latter being negligible; (3) young adult mice are more resistant to the toxic action of the drug than very young or old mice; (4) tumour regression is more obvious in some strains of mice than in others.

The idea of trying colchicine in the treatment of malignant growth is based on interference with cell division. The drug is believed to bring into mitosis all cells which are in karyokinetic imminence, and stops them at this stage (Bastenie and Zylberszac[6]). From consideration of this interference of colchicine with cell division we were led to investigate whether it exercises a similar action on bacteria; and because colchiceine is known to be less toxic than colchicine it was included in our work.

In Vitro Tests

Bacteriostatic Action. Varying dilutions of the two alkaloids in broth, sugar peptone water media, and agar were made, and the media inoculated with the following organisms: *Staphylococcus aureus, Gaffkia tetragenus, Bacillus anthracis, Bacillus subtilis, Mycobacterium phlei, Corynebacterium hofmanni, Salmonella typhi, Salmonella paratyphi A, Shigella flexneri, Shigella schmitzi, Escherichia coli, Vibrio cholerœ, Pseudomonas æruginosa,* and *Klebsiella pneumoniæ.* The inoculum used with the liquid medium consisted of 0·002 ml. of a 24 hours broth culture added to 100 ml. of medium. The dilutions of the alkaloids used were from 1/250 to 1/3000. For the agar medium a standard loop measuring 2 mm. in diameter was used for inoculation. The media were incubated at 37°C. for 24 hours and the results shown in Table I, were obtained. Colchicine was shown to be bacteriostatic to *Staph. aureus, Gaf. tetragenus, B. anthracis, M. phlei, V. cholerœ* and *C. hofmanni* in a dilution of 1/250 and colchiceine was shown to be bacteriostatic to *Salm. typhi, Salm. paratyphi A, Shig. schmitzi, Esch. coli* in a dilution of 1/250, to *Gaf. tetragenus, B. anthracis, B. subtilis, C. hofmanni* and *Shig. flexneri* in a dilution of 1/500 and to *Staph. aureus, M. phlei* and *V. cholerœ* in a dilution of 1/1000. Consequently colchiceine, the less toxic of the two alkaloids, is a more powerful bacteriostatic agent.

On examining the morphology of the organisms which grew in the presence of a sub-bacteriostatic concentration of colchicine and colchiceine, it was observed that they were slightly swollen, somewhat like penicillin-sensitive organisms grown in the presence of a sub-bacteriostatic concentration of this antibiotic.

Bactericidal Action. The organisms were exposed to the action of varying dilutions of the two alkaloids for varying periods, from half an hour to 24 hours, and sterility tests were subsequently made; no bactericidal action was detected.

To investigate whether sulphonamides and penicillin have a synergetic action on these alkaloids the following experiments were carried out. The minimal bacteriostatic concentration for each of the substances was determined, and by fixing one of them and varying the other it was

TABLE I

BACTERIOSTATIC ACTION OF COLCHICINE AND COLCHICEINE

Concentration of alkaloid	1/250	1/500	1/1000	1/3000	1/250	1/500	1/1000	1/3000	C.
Staphylococcus aureus...	−	+	+	+	−	−	−	+	+
Gaffkia tetragenus	−	±	+	+	−	−	±	+	+
Bacillus anthracis	−	+	+	+	−	−	+	+	+
Bacillus subtilis	±	+	+	+	−	−	±	+	+
Mycobacterium phlei	−	+	+	+	−	−	−	+	+
Corynebacterium hofmanni	−	+	+	+	−	−	+	+	+
Salmonella typhi	+	+	+	+	−	±	+	+	+
Salmonella paratyphi A	+	+	+	+	−	+	+	+	+
Shigella flexneri	+	+	+	+	−	−	+	+	+
Shigella schmitzi	+	+	+	+	−	±	+	+	+
Escherichia coli...	+	+	+	+	−	+	+	+	+
Vibrio choleræ	−	+	+	+	−	−	−	+	+
Pseudomonas æruginosa	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniæ	+	+	+	+	+	+	+	+	+

+ = growth. − = no growth. ± = scanty growth.

found that the minimal bacteriostatic dose of the latter was reduced. Thus growth could be inhibited by dissolving in the medium of sub-bacteriostatic doses of two of the substances, e.g., colchiceine and penicillin. This shows that a certain degree of synergism between these alkaloids, on the one hand, and sulphonamide compounds and penicillin, on the other.

In Vivo Tests

Toxicity. Before testing the alkaloids for protective action their toxicity was determined on guinea-pigs, weighing on the average 500 g., by intramuscular injection. The LD100 for colchicine was found to be 1·2 mg/kg. of body weight, and the corresponding dose of and for colchiceine 50 mg. Both alkaloids produced severe diarrhœa and vomiting, and these symptoms were invariably followed by death.

In view of the high toxicity of colchicine it was decided to test the antibacterial action of colchiceine only. For this guinea-pigs infected with *Staph. aureus*, which was one of the most sensitive organisms, were used. 3 animals were infected with a lethal dose of this organism by intraperitoneal inoculation. Simultaneously with the infection each animal received an intramuscular injection of 15 mg. of colchiceine in aqueous solution. 3 other animals were similarly infected but left without treatment. The latter died, while one of the treated animals survived. On

account of shortage of colchiceine the experiment could not be made on a larger number of animals, nor could the effect of treatment before and after infection be assessed. Consequently no definite conclusion can be drawn as to the effect of colchiceine in overcoming infection with *Staph. aureus*. Further it might have been advisable to try the effect of this relatively non-toxic alkaloid on malignant cells, but again shortage prevented the performance of this important experiment which, to our knowledge, has not been made before. Several attempts were made to procure that alkaloid from well known firms; but we failed to do so. Apparently chemical factories have ceased to take interest in this alkaloid which is hardly ever used in ordinary therapeutics.

Discussion

Colchicine has received some attention on account of its inhibitive action on cell division, and the prospect of using it in the treatment of malignant disease. Amoroso observed that the alkaloid produced regression of tumours in mice, and found it to be effective in the treatment of a spontaneous tumour in a dog. This led to further investigations by Ludford and others on the effect of this alkaloid on cell division in normal embryonic tissues as well as malignant tumours. The results indicate that inhibition of cell division is not limited to malignant growth but also involves normal tissues, and that the quantity needed for the destruction of tumour cells is not much less than the toxic dose. Thus Seed, Slaughter and Limarzi[7], after investigating the action of colchicine in man and animals came to the following conclusion: "Although the rapidly growing cancer cells are much more susceptible to the poison, the concomitant general toxic effect is much too great to expect any curative effect."

It is this toxicity which makes us hesitant to hope for any practical therapeutic application of the antibacterial action demonstrated by the work described. Added to this is the fact that the antibacterial action of both alkaloids is of a low grade, a relatively high concentration being needed to inhibit bacterial growth. Fortunately, however, colchiceine which is the less toxic of the two alkaloids, has a somewhat higher antibacterial effect than colchicine; and from the few experiments on laboratory animals cited above one may be justified in entertaining the idea that possibly this alkaloid, either alone or together with penicillin or a sulphonamide compound, may, after further trial, prove of some therapeutic value in certain infections. To our knowledge it has never been used in ordinary therapeutics, although colchicine is extensively used in the treatment of gout in spite of its toxicity.

The relatively low toxicity of colchiceine may also justify its trial on cell division, normal or of malignant nature, an experiment which we could not make because of our inability to obtain a sufficient supply. It is interesting to note the occurrence of morphological changes in organisms growing in the presence of sub-bacteriostatic doses of either alkaloid, a phenomenon which is well known in connection with penicillin and some other chemotherapeutic agents.

From the laboratory point of view these alkaloids may perhaps be used for the preparation of some selective media since they seem to be more effective on Gram positive than on Gram negative organisms.

The mechanism by which these alkaloids interfere with bacterial division has not been worked out, but it is not unlikely that it does not differ from that involved in ordinary cell division, especially as in the light of recent knowledge, based on the use of electron microscopy, several species of bacteria seem to possess a nuclear structure not unlike that of ordinary somatic cells.

Summary and Conclusions

1. Colchicine and colchiceine, two alkaloids of *Colchicum autumnale* have some antibacterial action on *Staph. aureus, Gaf. tetragenus, B. anthracis, B. subtilis, M. phlei, C. hofmanni, Salm. typhi, Salm. paratyphi A, Shig. flexneri, Shig. schmitzi, Esch. coli, V. cholerœ, Ps. œruginosa, Kleb. pneumoniœ.*

2. Colchiceine, a decomposition product of colchicine, is less toxic than colchicine and is a more powerful antibacterial agent.

3. *In vivo* colchiceine has a doubtful protective effect on guinea-pigs against staphylococcal infection, but until the experiment is made on a larger scale no conclusion can be stated.

4. A certain synergetic effect seems to exist between colchicine and colchiceine on the one hand and penicillin and sulphonamide compounds on the other.

5. On account of the toxicity of these alkaloids, especially colchicine, little practical therapeutic value is expected from their use in the treatment of bacterial infection. It is possible, however, by taking advantage of their selectivity, that they might prove useful in the preparation of certain selective media.

6. The trial of the effect of colchiceine on tumour cells is suggested.

References

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