THE ACTION OF COLCHICINE IN ARRESTING EPIDERMAL MITOSIS

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(With Two Text-figures)

I. INTRODUCTION

In the foregoing paper (Bullough, 1949*b*) a doubt is expressed concerning the value of colchicine for collecting in the metaphase all those mitoses which normally occur during a 12 hr. period. It is probably true to say that most studies of animal mitosis made with the help of colchicine have been planned on two assumptions, namely that, within reasonable limits, the drug arrests every mitosis reaching the metaphase, and, equally important, that it neither increases nor decreases the number of resting cells entering mitosis. Following the pioneer work of Dustin (1934), Lits (1934) and others, these reasonable limits have been defined (see Allen, 1937) as a dose of 0·1 mg. given subcutaneously in water or normal saline and allowed to act for a period of $9\frac{1}{2}$ hr. With smaller doses only a partial stoppage of mitosis is achieved; with larger doses the resting cells are prevented from entering the prophase; and with longer periods the number of arrested mitoses does not increase because there is a complete stoppage of all cell division.

By means of the earclip technique (Bullough, 1948) it is now possible to test these various assumptions and conclusions, and the results of preliminary experiments to this end are recorded below. Each experiment involved a comparison between two groups of ten male mice of identical ages. The animals of one group were each injected subcutaneously with 0·1 mg. of colchicine dissolved in 0·25 c.c. of water, while those of the other each received 0·25 c.c. of water as a control. At the time of the injection, and at 2 hr. intervals thereafter, an earclip was removed from each animal by means of a conchotome. The last earclips were taken 12 hr. after the time of injection. The clips were fixed in alcoholic Bouin, cut into sections 7μ thick, and, after staining, the mitoses were counted in unit section lengths of 1 cm. The method of assessing the degrees of mitotic activity was the same as that described in the foregoing paper.

II. OBSERVATIONS

Each of the first two groups of mice consisted of ten 3-month-old Kreyberg white label males. They were injected at 08.00 hr., and they gave the results recorded below and in Fig. 1.

While the control animals show the variations typical of this part of the diurnal cycle with a peak of activity at about 14.00 hr. (Bullough, 1948), it is evident that the colchicine-injected animals developed most of their mitoses before that time.

A comparison between the two groups of figures shows that at 14.00 hr. the number of arrested mitoses in the colchicine-injected animals should not have been less than 7, whereas in fact it was observed to be only 4·5. It is therefore evident that some time between 12.00 and 14.00 hr. the colchicine had begun to depress the number of resting cells which were entering the prophase. However, a few prophases were still to be seen at 14.00 hr. so that in all probability cell division was not entirely inhibited until after this time. After 14.00 hr. no more prophases were seen in the colchicine-injected animals although they continued to be observed in the control animals.

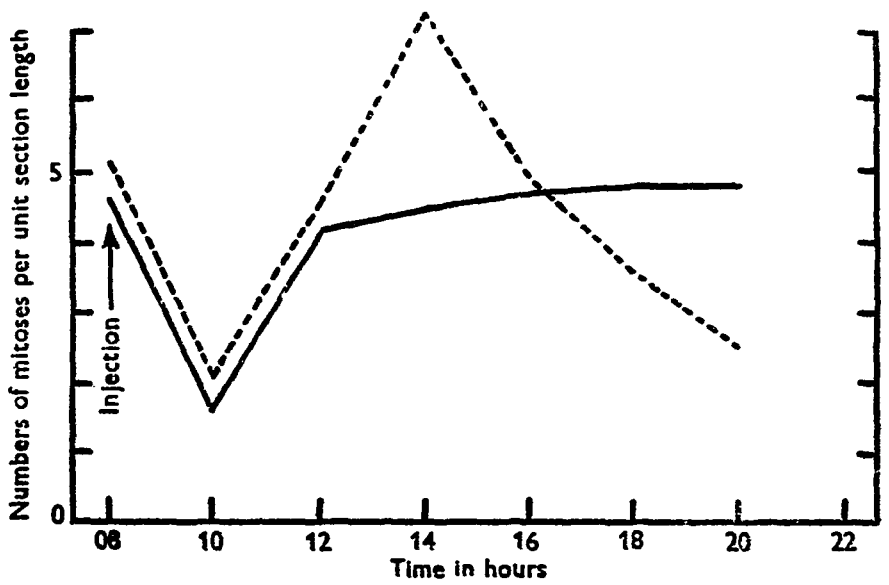

Fig. 1. The variations in the mitotic activity of the ear epidermis of mice injected at 08.00 hr. with colchicine dissolved in water (solid line) and with water alone (broken line).

Table 1. *The average numbers of mitoses present per unit length* (1 *cm.*) *of sections of the ear epidermis of adult male mice injected at* 08.00 hr.

Time of day	Mice injected with colchicine in water	Mice injected with water alone
08.00	4·6 ± 0·32	5·1 ± 0·25
10.00	1·7 ± 0·14	2·0 ± 0·39
12.00	4·2 ± 0·18	4·6 ± 0·23
14.00	4·5 ± 0·16	7·3 ± 0·30
16.00	4·7 ± 0·19	4·9 ± 0·19
18.00	4·8 ± 0·29	3·7 ± 0·23
20.00	4·8 ± 0·21	2·7 ± 0·21

It was also interesting to notice in the colchicine-injected mice that, while at 10.00 hr. only pro- and metaphases were to be found, at all subsequent hours both ana- and telophases were also present. Thus it was evident that many of the divisions succeeded in passing the metaphase, though whether any were able to reach the resting stage once more could not be determined.

An important practical consideration arises from these conclusions. It is obvious that if the drug is allowed to act for periods of up to $9\frac{1}{2}$ or 12 hr., the number of mitoses observed will depend not on the degree of mitotic activity normally occurring during these periods, but on the degree of mitotic activity occurring during the

first 5 hr. only. Thus, at least as regards the ear epidermis, the maximum time which an injection of 0·1 mg. of colchicine should be allowed to act in an adult mouse is 5 hr., or if it is allowed to act longer, then the result obtained should be regarded as referring only to the first 5 hr. period.

The practical importance of this is demonstrated in Table 2, which is based on the results of an experiment in all ways similar to the first except that the injections were given at 10.00 instead of 08.00 hr. These results are also expressed graphically in Fig. 2.

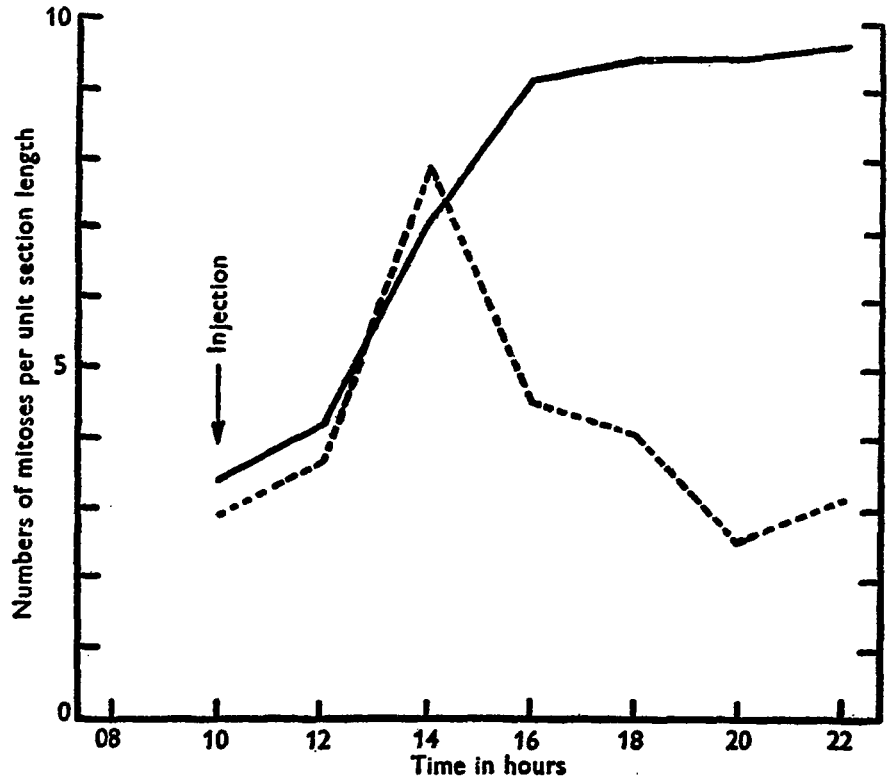

Fig. 2. The variations in the mitotic activity of the ear epidermis of mice injected at 10.00 hr. with colchicine dissolved in water (solid line) and with water alone (broken line).

Table 2. *The average numbers of mitoses present per unit length* (1 *cm.*) *of sections of the ear epidermis of adult male mice injected at* 10.00 *hr.*

Time of day	Mice injected with colchicine in water	Mice injected with water alone
10.00	3·4 ± 0·27	2·9 ± 0·21
12.00	4·2 ± 0·24	3·6 ± 0·37
14.00	7·1 ± 0·31	7·9 ± 0·25
16.00	9·3 ± 0·33	4·6 ± 0·19
18.00	9·5 ± 0·16	4·2 ± 0·19
20.00	9·5 ± 0·52	2·7 ± 0·20
22.00	9·7 ± 0·55	3·2 ± 0·15

Once again the colchicine only arrested mitoses in or after the metaphase during a period of about 5 hr. After 6 hr. no more prophases were discovered, and it is probable that no more resting cells were entering into division. However, since in this experiment the first 5 hr. coincided exactly with the rise in the mitosis rate which is normally associated with the early afternoon sleep period, twice as many divisions were arrested as in the first experiment.

A comparison between the control mice in the two experiments shows that the

number of divisions normally occurring in the two 12 hr. periods 08.00–20.00 hr. and 10.00–22.00 hr. was about the same. In both cases the average figures add up to about 30. Thus it was merely the 2 hr. difference in the time of injection which was responsible for the apparent doubling of the mitosis rate in the colchicine-injected animals of the second experiment. This result can only throw doubt on those published accounts of mitotic activity in which the period of colchicine action has been longer than 5 hr., and in which no account has been taken of the diurnal cycle.

During this work it was accidentally observed that the administration of 0·1 mg. of colchicine to an adult mouse induces a severe depression of the blood-sugar level. This fact does not appear to have been observed before, and the reason for it is unknown. Following the discovery of the direct connexion existing between the availability of sugar and the rate of mitosis (Bullough, 1949*a*), it was suspected that the depression of mitotic activity after the first 5 hr. period might be connected with this depression in the blood-sugar level. Consequently a series of observations was made on the effect of colchicine on blood sugar.

The same technique as before was adopted, the experimental mice being injected at 08.00 hr. with 0·1 mg. of colchicine in 0·25 c.c. of water, and the control mice with 0·25 c.c. of water alone. The technique by which the samples of blood were taken and their sugar content estimated is described by Bullough (1949*a*), and once again it is a pleasure to mention the assistance received from Dr A. Jordan who arranged for the blood-sugar estimations to be made in the laboratories of the Sheffield Royal Infirmary. Clearly the drop in the blood-sugar concentration, very

Table 3. *Variations in the blood-sugar concentration following an injection of colchicine at* 08.00 *hr.*

Time of day	Mice injected with colchicine in water		Mice injected with water alone	
	No. of observations	Blood-sugar level in mg. per 100 c.c.	No. of observations	Blood-sugar level in mg. per 100 c.c.
08.00	10	148·3 ± 5·12	10	151·1 ± 5·07
14.00	8	168·0 ± 4·71	10	147·9 ± 4·13
20.00	20	115·9 ± 3·93	10	182·8 ± 4·76

apparent after 12 hr., had not developed after only 6 hr., and indeed there are signs that an actual rise had occurred at that time. It follows that the depression of mitotic activity, which begins about 5 hr. after the injection of colchicine, may well be independent of changes in the blood-sugar concentration.

III. CONCLUSIONS

1. It is evident from the above experiments that for a period of about 5 hr. after the injection of 0·1 mg. of colchicine into an adult mouse there is no discernible change from the normal in the epidermal mitosis rate, and that during this period all, or most, of the epidermal mitoses are arrested in some stage after the prophase.

After this 5 hr. period the colchicine inhibits mitotic activity by preventing any more resting cells from entering the prophase.

2. During the first 5 hr. the arresting action of the colchicine is not felt until a division reaches the metaphase. However, considerable numbers of divisions succeed in passing this stage to reach the ana- and telophases, and it is possible that some of these divisions are completed.

3. After the first 5 hr. the mitosis inhibiting effect of colchicine is associated with severe nervous depression which ultimately may end in death. It is possible that both effects may be dependent on the formation of a toxic oxidation product, oxydicolchicine (Goodman & Gilman, 1947). It is also noted that colchicine depresses the blood-sugar concentration, but that this depression does not become apparent until after 6 hr. have passed.

4. The practical importance of these results is considerable. It is evident, at least in the case of the ear epidermis, that for a study of normal mitotic activity colchicine must not be allowed to act for more than about 5 hr. The period of $9\frac{1}{2}$ hr. suggested by Allen (1937) is almost twice too long, and, due to the complication of the diurnal cycle, the use of such a long period may yield the most anomalous results.

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