# REESTABLISHMENT OF CELL DIVISION AFTER REACTIVE INHIBITION BY DEPRESSION OF THE SYMPATHETICOADRENAL SYSTEM

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The existence in the body of factors and systems regulating the processes of proliferation of cells in various tissues and organs has recently been proved by a number of special investigations, including some by us [1-8, 10]. It has been established that the widest variety of stimuli in the external environment, which produce excitation of the body and an increase in the tone of the nervous sytem, alter the intensity of proliferation of the tissue cells. Under these circumstances the mitotic activity usually falls; reactive inhibition of cell division is observed.

Friedenwald and Buschke [10], who first discovered the phenomenon of reactive inhibition of the processes of cell division, put forward the view that this reaction is effected through a humoral mechanism. The experimental observations of these workers, and the later research by Bullough [9], the author [4, 6], I. A. Alov [1] and others showed that an analagous depression of cell division is also observed after injection of adrenalin into the animal. On the other hand, it was shown in work by I. A. Alov [1] and A. K. Ryabukha [6], that reactive inhibition of cell division is not observed or is considerably weakened in adrenalectomized animals.

It may therefore be regarded as proved that the mechanism of reactive inhibition of the proliferation of tissue cells is a neurohumoral one, and that one of its main links is the adrenals.

Continuing the study of the laws of cell division and the relationship between the processes of division and various physiological conditions of the body, we undertook an investigation of the changes in cell division during the frequent and prolonged action of stimuli on the animal when the nervous system was either in a condition of excitation or of depression. The question arose, what takes place in these conditions in the processes of cell division; how long can reactive inhibition be maintained; do not the regulating mechanisms become exhausted, and if so, how is this reflected in cell division; do the cells themselves show adaptation to the humoral factor inhibiting division, and so on.

## EXPERIMENTAL METHOD

We carried out experiments on white mice of the same age and sex. Prolonged stimulation of the animals was carried out by a weak electric current of the minimum voltage to which they reacted, in a special chamber with a metal floor which was included in the circuit. Stimulation was by means of isolated impulses of 3-4 sec duration, at intervals of 6-8 sec, for 4 hr 30 min, 7 and 9 hr. The circuit was opened and closed automatically.

In other experiments we used adrenalin as a long acting stimulus. The hormone was given as a 1:20,000 solution, in a dose of 0.2 ml per injection. In view of the instability of the preparation, it was injected repeatedly into the animals: each hour for 4 and 7 hours, every 2 hours for 10 hours, and every 3 hours for 24 hours.

Remembering the natural daily variations of mitotic activity, a separate control was provided for each experiment. The control animals were killed at the same times as the experimental animals. The test object was the stratified epithelium of the cornea of the eye. The material was fixed in Bouin's fluid. Total preparations of the cornea were obtained (stained by Caracci's method and by Yasvoin's iron-hematoxylin method); some of the material was embedded in paraffin wax for the preparation of histological sections (stained with hematoxylin-eosin).

To determine the mitotic activity, 100 fields were examined in each cornea from the animal and the topographical pattern of the mitoses was compared; we took into consideration both the total number of dividing cells and their phase relationships.

## EXPERIMENTAL RESULTS

In our previous investigations [3, 6] we showed that transient, mechanical painful stimulation of the animal or the action of an electric current on the animal for 15 to 30 min leads to severe changes in the phase relationships of mitotic division; under these circumstances the early stages of mitosis as a rule disappear. A shift is observed in the phases also after action on the animal for 5 minutes, if the material is not fixed immediately but after a short time, e.g. 30-40 min.

Increase in the length of the time of action to 1 - 2 hours causes the reactive inhibition of cell division to extend to all the phases of mitosis. Stimulation for 60 minutes is usually enough to cause almost complete suppression of the processes of division. Mitotic activity falls by 91-93% by comparison with the controls, and only solitary dividing cells remain. Further increase in the period of action to 2-3 hours causes only little change in the reactive inhibition of cell division. The processes of cell proliferation are practically completely inhibited; nevertheless a very small percentage of mitoses remains even under these conditions.

The solitary dividing cells still do not disappear after stimulation for 4 hours; in fact in this case some increase in the number of mitoses may even be observed, mainly in the earlier stages of division — the prophase. This chance observation was confirmed by a special investigation. In all experiments without exception in which stimulation lasted 4 hr 30 min we observed a shift in the opposite direction in the phases of mitotic division.

As seen from Table 1, in which are shown the results of one of these experiments (animals were stimulated by an electric current for 4 hr 30 min), the mitotic activity was at the rate of 10% of the initial level, and the proportion of early stages of division was relatively high.

Ana-and telophases are almost absent; this suggests that division was completely suppressed not long before the end of the experiment. The number of prophases reaches 34% of the control figure, and this undoubtedly is an indication of the incipient increase in mitotic activity in the epithelium.

TABLE 1

Reestablishment of Mitotic Activity in Animals During Prolonged Stimulation

| Duration of stimulation | Number of corneas | Phase of mitosis | | | | Total number of mitosis |
|---|---|---|---|---|---|---|
| | | P* | M | A | T | |
| Control | 8 | 29 | 27 | 17 | 43 | 116 |
| 4 hr 30 min | 6 | 10 | 1.5 | 0 | 0.7 | 12 |
| %,control fig. | — | 34 | 5 | 0 | 1.6 | 10 |
| Control | 6 | 20 | 30 | 18 | 39 | 106 |
| 7 hr | 6 | 16 | 7 | 6 | 9 | 39 |
| %, control fig. | — | 80 | 23 | 33 | 23 | 37 |

*Here and in the following tables P, M, A and T indicate pro-, meta-, ana- and telophase.

TABLE 2

Mitotic Activity of the Corneal Epithelium After a Single Injection of Adrenalin to Animals Subjected to Prolonged Stimulation

| Time elapsing after injection of adrenalin | Number of corneas | Phases of mitosis | | | | Total number of mitosis |
|---|---|---|---|---|---|---|
| | | П | М | А | Т | |
| Control | 6 | 20 | 30 | 18 | 39 | 106 |
| 40 minutes | 4 | 2 | 0 | 1 | 9 | 12 |
| 1 hr 30 min | 6 | 2 | 0 | 0 | 1 | 3 |

TABLE 3

Suppression of the Restoration of Cell Division by Adrenalin on Its Re-establishment After Previous Inhibition

| Duration of stimulation | Number of corneas | Phases of mitosis | | | | Total number of mitosis |
|---|---|---|---|---|---|---|
| | | P | M | A | T | |
| Control | 6 | 15 | 15 | 10 | 24 | 64 |
| 9 hours | 6 | 17 | 11 | 5 | 11 | 45 |
| % control fig | — | — | — | — | — | 70 |
| 9 hours + adrenalin | 6 | 0 | 0 | 0 | 5 | 5 |

In another series stimulation of the mice continued for 7 hours. Under these conditions clear signs of exhaustion of the animals and depression of their nervous system developed, although the stimuation was applied in the form of separate short impulses with longer pauses in between. Table 1 shows that, in spite of the continuing action of the stimulus, usually leading to sharp suppression of cell division, the process of reestablishment of mitotic activity progressed further.

The number of mitoses in the corneas of the experimental animals amounted at this time to 37%, i. e. it was still considerably lowered. The intensity of division continued to rise slowly; in this case, however, there was a preponderance of prophases, the number of which reached 80% of the control figure. Similar results were also obtained in the other experiments.

From an analysis of our findings it can be deduced that the processes of proliferation of cells which are subjected to inhibition as a result of prolonged stimulation are reestablished despite the continuing stimulation. Restoration begins after approximately 4 hours; under these circumstances, we may believe, not all the "inhibited" cells begin to undergo division at the same time, otherwise the reestablishment of the initial level of mitotic activity would be observed one hour after the beginning of restoration, since the duration of mitotic division in the corneal epithelium is approximately 1 hours. In fact, as may be seen from the results shown, the process of restoration lasts for much longer. It is not impossible that under these circumstances the time taken for mitosis itself in the experimental animals should also be lengthened to some extent.

What is the cause of this restoration of the processes of proliferation of the cells? It may be due to two factors. First, it may be due to adaptation of the cells to the factor which prevents the transition to mitosis. The second possible cause is depression or exclusion of the neurohumoral mechanism of reactive inhibition of division.

In order to elucidate this problem we carried out the following experiments. Animals were exposed to stimulation for 6-7 hours, after which they were at once given an injection of adrenalin. Some of the mice stimulated for 6 hours were killed 40 minutes after the injection, while the animals of the other group were

stimulated for 7 hours and received two injections of the hormone: immediately after stimulation and after another 30 minutes; the material was fixed 1 hr 30 min after the first injection.

TABLE 4

Mitotic Activity of the Corneal Epithelium after Repeated Injection of the Animal with Adrenalin (5 injections) Over a Period of 4 hr 30 min

| Observation | Number of corneas | Phases of mitosis | | | | Total number of mitoses |
|---|---|---|---|---|---|---|
| | | P | M | A | T | |
| Control | 6 | 18 | 17 | 8 | 32 | 75 |
| Experiment | 6 | 0 | 0 | 0 | 0 | 0 |

As seen from Table 2, the mitotic activity in the corneal epithelium of the animals killed 40 minutes after injection of adrenalin is considerably reduced, and the total number of mitoses is one third that of animals exposed to the same stimulation (compare Table 1) without the subsequent injection of adrenalin. The early phases of mitosis are almost completely absent; the telophases are still comparatively numerous, since after 40 minutes they are unable to proceed into the resting stage. Such a relationship between the phases indicates that before injection of adrenalin the mitotic activity was at a very high level, i. e. reestablishment of the processes of division had taken place after their inhibition.

In the animals of the second group, killed 1 hour 30 minutes after the first injection of adrenalin, total suppression of division is observed.

Thus the processes of cell proliferation, reestablished during prolonged stimulation of the animal, may be suppressed secondarily by injection into the animal of the hormone of the adrenal medulla. It is, therefore, not a question of adaptation of the cells, nor yet that the cells cease to react to adrenalin, which is secreted in increased amounts during excitation of the animal. The cause of the reestablishment of cell division during prolonged stimulation of the animal is depression of the sympatheticoadrenalin system. In the conditions of our experiments the adrenals ceased to react with increased secretion of adrenalin, and the phenomenon of reactive inhibition of mitotic activity gradually disappeared. Continuing stimulation did not produce suppression of cell division, although the animals reacted as before to the stimulus. This conclusion is confirmed by the next experiments (Table 3). The phenomenon described may be regarded, it seems, as regulatory adaptation on the part of the animal.

We were interested to ascertain whether the mitotic activity is completely restored to its original level during continued stimulation. Animals were stimulated for 9 hours, and some of them killed immediately afterwards. From Table 3 it is seen that at this time a fairly large number of mitoses is observed in the corneas of the experimental animals; their total number still does not reach the control figure, but mitotic activity is already 70% of this value and it is clear that it will rise to the original level—this is shown by the phase relationships.

The animals of the second group, which were stimulated for the same length of time, were injected with adrenalin, and then killed 40 minutes later. As it was to be expected, secondary suppression of cell division was observed in the epithelium of these animals.

In connection with the results obtained, the question naturally arises whether restoration of the processes of division will be observed also after the prolonged injection of adrenalin into the animals; or whether, in spite of the possible depression of the nervous system under these conditions, cell division will be suppressed for a long time. Under these circumstances, what morphological changes may take place in the tissues?

In order to elucidate these problems we performed a series of experiments. Animals were injected subcutaneously with 0.2 ml of a 1:20,000 solution of adrenalin repeatedly over a long period of time. In Table 4 are shown the results of these observations. The hormone was injected 5 times in the course of 4 hours, i. e.

TABLE 5

Prolonged Inhibition of the Processes of Division by Repeated Injection of Adrenalin into the Animal

| Duration of injection | Time of fixation after last injection | Number of corneas | Phases of mitosis | | | | Total Number of mitosis |
|---|---|---|---|---|---|---|---|
| | | | П | М | А | Т | |
| For 7 hours (every hour) | Control | 6 | 32 | 29 | 11 | 24 | 96 |
| | After 45 minutes | 6 | 1 | 0.5 | 0 | 0 | 2 |
| | After 2 hours | 6 | 4 | 2.5 | 1.5 | 1 | 9 |
| For 10 hours (every 2 hours) | Control | 6 | 43 | 63 | 26 | 39 | 171 |
| | After 1 hour | 4 | 2 | 3 | 1 | 0 | 6 |
| | After 2 hours | 4 | 6 | 7 | 5 | 8 | 26 |
| For 24 hours (every 3 hours) | Control | 4 | 24 | 25 | 11 | 27 | 87 |
| | After 1 hour | 2 | 2 | 1 | 0 | 1 | 4 |
| | After 3 hours | 4 | 37 | 22 | 19 | 23 | 101 |

once every hour. The material was fixed 30-45 min after the last injection. As can be seen, the mitotic activity was completly suppressed. There were no signs of restoration of division. It is interesting to recall that at this period of stimulation of the animals with the electric current, an obvious reverse shift was observed in the relationships of the phases of mitosis (see Table 1).

In another series of experiments adrenalin was injected over a period of 7 hours, also at intervals of 60 min (Table 5). When the material was fixed 45 min after the last injection, as in the previous case total suppression of division was observed. Some mice were killed 2 hr after the last injection. In the corneas of these animals an increase was noted in the number of early stages of division. At this time, therefore, the action of adrenalin has already come to an end.

As we know, adrenalin is an unstable compound; it is rapidly inactivated in the body as a result of enzymic oxidation. Because of this, we also injected animals with adrenalin over a longer period of time—10 hours (every 2 hours) and even for 24 hours. It should be mentioned that the mice tolerated the repeated injections of adrenalin very well indeed; none of the animals died as a result.

As may be seen from Table 5, as a result of injection of the animals with adrenalin the processes of cell division are not restored during the whole period of injection of the hormone, namely 10 hours. In the case of painful stimulation of the mice, at the 9th hr from the start of the experiment (see Table 3) a well marked increase in mitotic activity is observed, reaching 70% of the original level.

Tissue cells thus do not show adaptation to adrenalin, and even after repeated injections of this hormone they still retain the power to react with inhibition of division. In the last experiment (as in the previous one), when the material is fixed 2 hours after the last injection, an accumulation of mitoses is observed. Hence the state of inhibition of division lasts no longer than 2 hours after the injection of the hormone has come to an end. Investigation of the mitotic activity in the corneal epithelium of animals in which adrenalin was injected every 3 hr for 24 hr shows convincingly that (see Table 5) in this case cell division was suppressed. In mice killed after 1 hour, mitoses were almost absent. However, when the material was fixed 3 hr after the last injection, the number of dividing cells was slightly greater even than the number in the control animals (116%).

Transverse sections of one of the corneas of each animal in the last series (both experimental and control) were examined histologically. Under these conditions of activity on these animals, no essential morphological changes in the corneal tissues could be found. However, the possible suppression of cell division in the tissues for a long period of time, with consequent interference with the course of physiological regeneration during frequent or prolonged stimulation of the animal, gives grounds for the suggestion that morphological changes in the tissues and organs are also possible under these circumstances. This problem clearly has a bearing on practical medicine and calls for special study.

## SUMMARY

In prolonged stimulation of mice by weak electric current the reactive inhibition of the mitotic activity in the corneal epithelium ceases. However, in 4 hours it reappears notwithstanding the continuing stimulation. Adrenalin administration again depresses the mitosis. If this hormone is administered every 2-3 hours it is possible to inhibit the tissue cells mitosis for hours (even as long as 24 hours). There was no adaptation of cells to adrenalin.

Thus the cause of the reestablishment of mitotic activity in prolonged action of the current consists in depression of the neurohumoral mechanism responsible for the inhibition of mitosis, mainly of adrenal system.

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