EFFECT OF ADRENAL HORMONES AND ADRENALECTOMY ON MITOTIC ACTIVITY*

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ALTHOUGH considerable work has been done on the effect of exogenous chemical substances which inhibit or stimulate mitotic activity, comparatively little work has been done on the effect of endogenous substances such as hormones. The effects of testosterone and oestrogens have been studied and these have been shown to stimulate mitotic activity (Bullough and van Oordt, 1950; Bullough, 1946, 1950), but the effect of the adrenal hormones had not been investigated when this work was started.

That such a study might prove worth while became apparent on reviewing the effects of adrenocorticotrophic hormone of the anterior pituitary (ACTH) and cortisone on tissue growth observed by other workers. Thus it has been shown that ACTH, which induces glucocorticoid hormone secretion, suppresses the growth of epidermis, sebaceous glands and hair (Baker, Ingle, Li and Evans, 1948) and that cortisone itself reduces the size of sebaceous glands (Castor and Baker, 1950). Further, it has been shown that ACTH causes involution of the lymph nodes (Baker, Ingle and Li, 1951; Feldman, 1951), while cortisone retards the growth rate of young rats (Wells and Kendall, 1940; Baker, 1950). Several recent studies have also indicated that the glucocorticoid hormones inhibit wound healing (Baker and Whitaker, 1950; Bangham, 1951; Billingham, Krohn and Medawar, 1951). The daily administration of cortisone delays the vascularization of a wound, weakens primary healing, depresses cellular proliferation and retards tissue reorganization. Green (1950) suggested that one possible way in which the adrenal hormones could produce such effects would be by depressing mitotic activity in the tissues.

Meanwhile a different approach to the problem began to suggest that the adrenal hormones might have an inhibiting effect on mitosis. Green and Bullough (1950) showed that following hind-limb ischaemia or the injection of adenosine triphosphate (ATP) there was an early and strong reduction, or complete suppression, of mitotic activity in the skin of the mouse. Further, it was demonstrated (Stoner and Green, 1950) that these stress-producing stimuli lead to a stimulation of the pituitary-cortical mechanism and the liberation of cortical hormones. It seemed likely therefore that the mitotic inhibition seen under these circumstances was produced indirectly as a result of the adrenal hormones, and it was decided to test this hypothesis by studying directly the effect of these hormones on mitotic activity in the mouse epidermis.

Bullough (1948*a*) devised a technique for studying mitotic activity in the ear epidermis of the mouse. He showed that there exists a clearly defined double diurnal cycle of mitotic activity in the epidermis of adult (3–4 months old) male

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mice, when these animals are kept at a temperature of 20° and fed every day between 9 and 10 a.m. Under these conditions periods of maximum mitotic activity were seen at 6 a.m. and 2 p.m. and periods of minimum activity at 10 a.m. and 8 p.m. Both Oritz-Picon (1933) and Carleton (1934) described cycles of mitotic activity in the epidermis, but they found respectively that the maximum occurred during the day and during the night. Cooper and Franklin (1940) reported that the greatest epidermal mitotic activity in their mice was seen at about 10 a.m. and Blumenfeld (1942) found the maximum at 12 noon in mice and at 9 a.m. in rats.

In view of these somewhat conflicting results it was deemed advisable to establish the normal mitotic cycle in the strains of mice and rats intended for use in later experiments, in a strictly controlled external environment. It appears that environmental factors can markedly modify the mitotic cycle and this was confirmed by many observations of our own. The normal mitotic cycle having been established the effect of adrenaline and cortisone was investigated, and it was found that they depress mitotic activity.

The effect of adrenalectomy was then investigated, once it seemed possible that the removal of the source of these mitotic depressant hormones might lead to increased mitotic activity. The possibility of complicating factors due to operative procedure was borne in mind. They might lead to the liberation of adrenal hormones and thus affect mitotic activity in the early stages of the experiment. There was also the possibility of compensating mechanisms following adrenalectomy which might affect the final picture. Hence the time elapsing between operative procedure and examination of the mitotic cycle might be of importance. Therefore the mitotic activity was studied at 48 hr., and 18 days after operation.

The results suggested that a compensating mechanism did arise. To test this further the effect of exercise, a stimulus releasing adrenaline, on the mitotic activity of intact, sham-operated and adrenalectomized mice was determined.

MATERIALS AND METHODS

The animals used were WLL mice, CBA mice and Wistar rats aged 3–4 months. These were maintained in a strictly controlled external environment. Cages housing the animals were kept in a constant temperature chamber maintained at 20°. They were fed on a diet of rat cubes, oats, cooked flaked maize, dog biscuits and cod liver oil regularly between 9.45 and 10 a.m. each day. Natural and/or electric light was provided from approximately 8 a.m. to 6 p.m. Only after they were stabilized in this manner for three weeks or more were they used for experiments.

The mitosis rate was measured in the ear epidermis by means of the ear clip technique (Bullough, 1948*a*). Small pieces of the ear were removed at intervals, fixed in Bouin's alcoholic fluid, embedded in wax and cut into sections 7 μ thick. All stages of mitosis were counted in unit section lengths of 1 cm. of the ear epidermis, and from each ear clip 10 such counts were made and the average taken.

Adrenaline hydrochloride (Parke Davis and Co.) was used as a 1 : 1000 solution in water. *Cortisone acetate* in the aqueous vehicle No. 1, supplied by Merck and Co. was used.

RESULTS

Normal Mitotic Cycle in Mice and Rats

Ten male mice of both WLL and CBA strains were used in experiments 1 and 2, 10 rats of the Wistar strain were used in experiment 3. Fig. 1, 2 and 3 illustrate mitotic cycles seen in the 3 experiments.

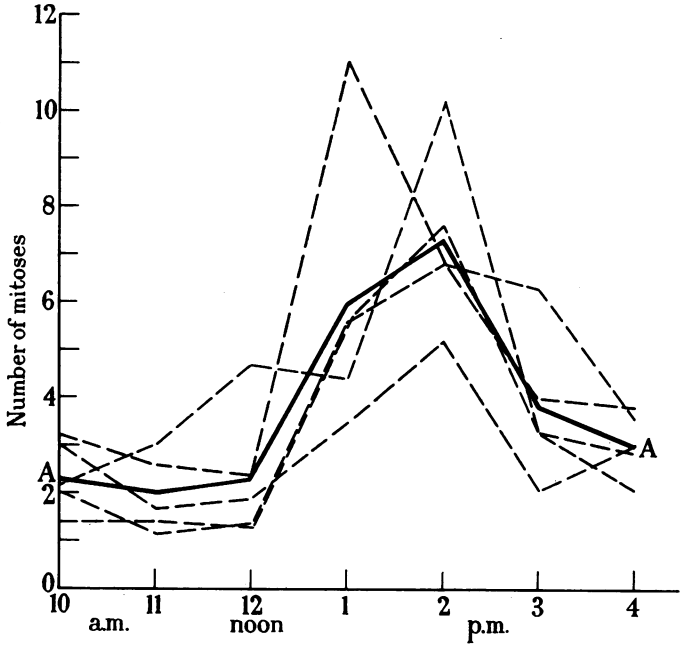

FIG. 1.—Normal mitotic cycle in WLL mice.
– – – – – – = Individual experiments.
A———A = Mean.

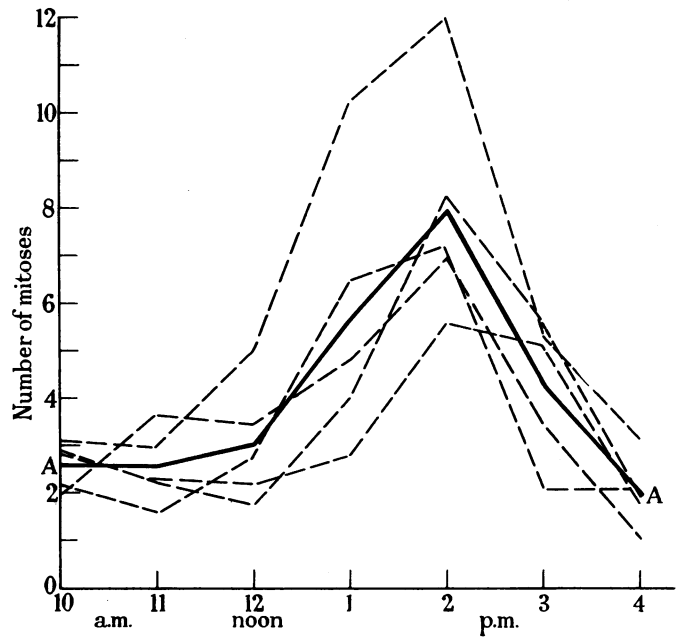

FIG. 2.—Normal mitotic cycle in CBA mice.
– – – – – – = Individual experiments.
A———A = Mean.

In spite of considerable individual variation the mean pattern and magnitude of the rise and fall of mitotic activity is very similar in all 3 groups. Thus at the peak period (2 p.m.) there were 7·3 mitoses per cm. in WLL mice, 8 in CBA mice and 6·5 in the rats.

These findings confirm Bullough's (1948*a*) results with WLL and CBA mice and further show that the pattern of mitotic activity in Wistar rats is very similar to that in mice.

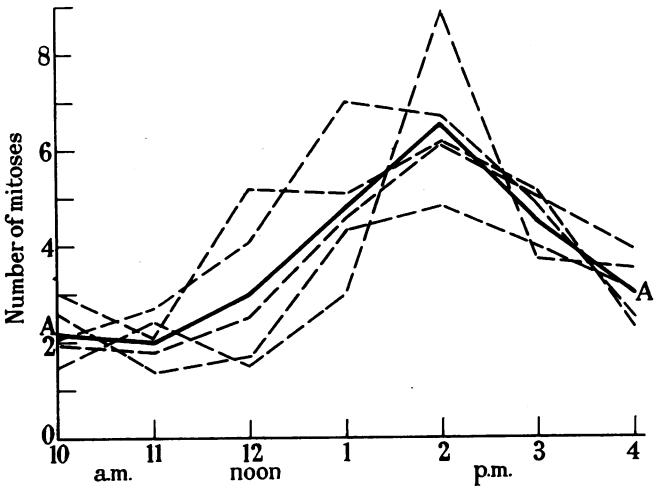

FIG. 3.—Normal mitotic cycle in Wistar rats.
– – – – – – = Individual experiments.
A———A = Mean.

Effect of Adrenaline on the Mitotic Cycle

Of 10 male mice of the WLL strain 5 received 0·04 mg./10g. mouse weight adrenaline subcutaneously, the injection being made immediately after the first ear clip at 10 a.m. Control animals received an equivalent volume (0·1 ml.) of 0·9 per cent saline.

The results (Fig. 4) show that adrenaline abolished the expected rise in the mitotic cycle. Thus at the peak period (2 p.m.) when the control animals showed a mean count of 6·8 mitoses per cm. the experimental animals showed only 0·2 mitoses and 2 hr. later the mean count had fallen to zero.

Effect of Cortisone

Of 10 male mice of the WLL strain 5 received cortisone, 2 mg./10g. mouse weight, subcutaneously, immediately after the first ear clip at 10 a.m. : 5 control animals received an equivalent volume of 0·9 per cent saline.

Results shown in Fig. 5 show that cortisone abolished the expected rise in the mitotic cycle. Thus at the peak period (2 p.m.) when the control animals showed a mean count of 7·3 mitoses per cm. the count in the experimental animals was 0·1 mitosis per cm. and 1 hr. later the mean count had fallen to zero.

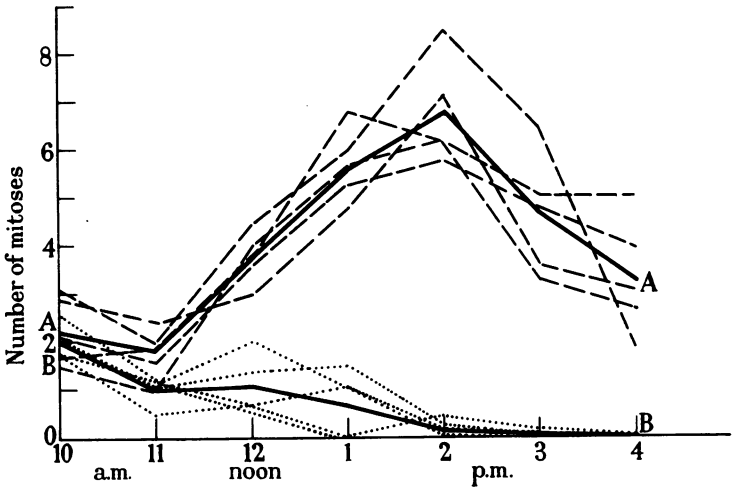

FIG. 4.—Effect of adrenaline on the mitotic cycle of WLL mice.
- - - - - - - = Individual controls.
A——A = Mean of controls.
. . . . . = Individual adrenaline-injected.
B——B = Mean of adrenaline-injected.

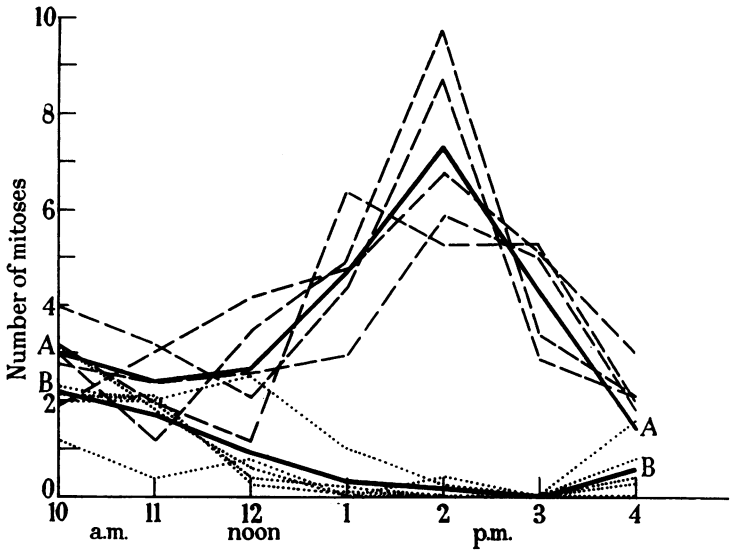

FIG. 5.—Effect of cortisone on the mitotic cycle of WLL mice.
- - - - - - - = Individual controls.
A——A = Mean of controls.
. . . . . = Individual cortisone-injected.
B——B = Mean of cortisone-injected.

Effect of Total Adrenalectomy and Sham Operation

Eight male CBA mice were used. In 4 mice both adrenal glands were removed under ether anaesthesia. After operation 1 per cent NaCl replaced ordinary drinking water. In 4 control animals a mock operation was performed. The technique was identical except that the adrenals were exposed to view but not removed. Forty-eight hours after operation ear clips were taken at hourly intervals from both groups, starting at 10 a.m. At the conclusion of the experiment the completeness of adrenalectomy was confirmed by killing the mice and examining the perinephric tissues macroscopically and microscopically.

Fig. 6 shows clearly that the mean pattern of mitotic activity differed from the normal in both control and experimental animals. In the controls mitotic activity

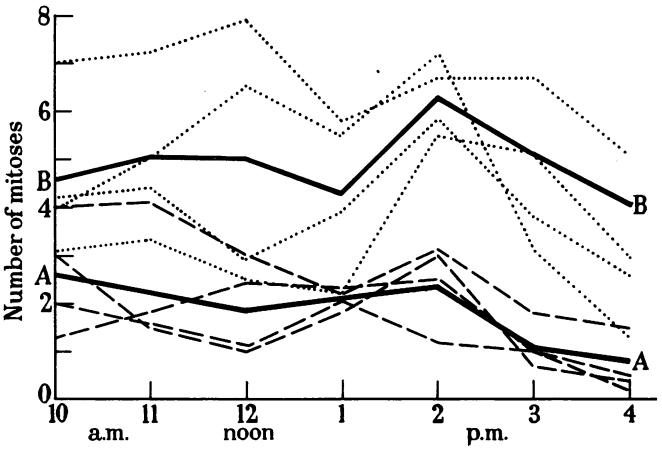

FIG. 6.—Effect of adrenalectomy on the mitotic cycle of CBA mice 48 hours after operation.

– – – – – – = Individual sham-operated.
A———A = Mean of sham-operated.
. . . . . = Individual adrenalectomized.
B———B = Mean of adrenalectomized.

was much depressed, this being particularly well demonstrated at the expected peak period (2 p.m.). In the adrenalectomized group, however, the peak of the curve is within normal limits although the rest of the points on the curve are somewhat elevated, suggesting that some stimulation of mitotic activity was occurring.

The next experiment was similar except that mitotic counts were made 18 days after operation. Fig. 7 shows that in both groups the pattern of mitotic activity is similar to that of normal animals.

Effect of Exercise in Normal and Adrenalectomized Rats

In this experiment there were 3 sham-operated, 6 adrenalectomized, and 3 intact control animals. Eighteen days after adrenalectomy, at 10 a.m., an ear clip was taken from all the animals in the various groups. All were then placed in a slowly revolving (80 rev. per hr.) cage driven by an electric motor. By this method the animals were prevented from going to sleep as they usually do during the course of an experiment, and were forced to change their position more or less

continuously. During the 6 hr. duration of the experiment it was estimated that the minimum distance which each rat had been forced to travel to maintain normal posture was approximately 480 yards (439 m.). Ear clips were taken at hourly intervals. Fig. 8 shows that there was a marked depression of mitotic activity in the intact and sham-operated controls while the adrenalectomized rats showed a fairly normal pattern.

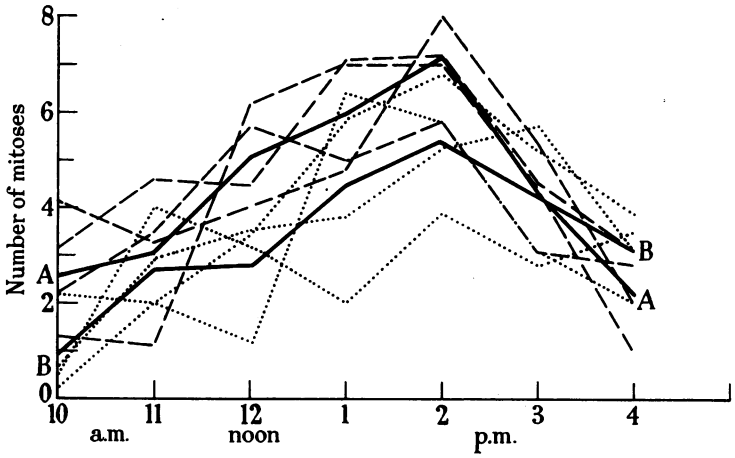

FIG. 7.—Effect of adrenalectomy on the mitotic cycle of CBA mice 18 days after operation.
– – – – – – = Individual sham-operated.
A———A = Mean of sham-operated.
. . . . . = Individual of adrenalectomized.
B———B = Mean of adrenalectomized.

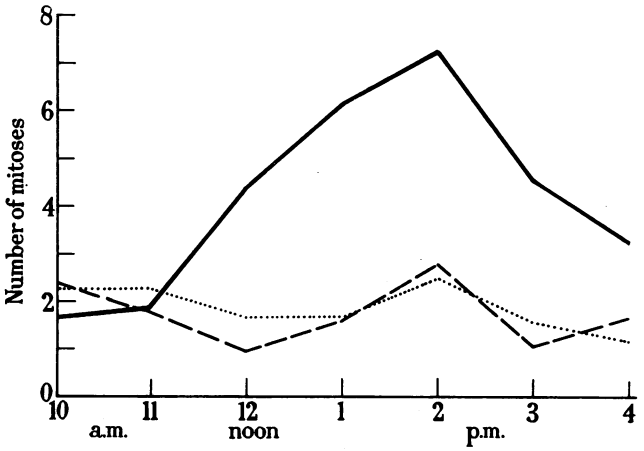

FIG. 8.—Effect of exercise on the mitotic cycle in adrenalectomized, sham-operated and normal Wistar rats.
——— = Adrenalectomized.
– – – – – – = Sham-operated.
. . . . . = Normal.

DISCUSSION

The normal mitotic cycle

The results show that mitotic activity in the epidermis of WLL mice, CBA mice, and Wistar rats is cyclical. Though there is a high degree of individual variation, maximum cell division is seen at approximately 2 p.m., and minimum at about 10 to 11 a.m. These results are similar to those of Bullough (1948*a*) for WLL and CBA mice, but also show that in Wistar rats the cycle is similar in magnitude and rhythm.

However, it must be stressed that this pattern of activity is seen only when the animals are maintained under strictly controlled environmental conditions. It would appear (Bullough, 1948 *a*, *b*) that the type of cycle common to any one colony is largely determined by the habits of resting and waking, and these will be determined by the routine of the laboratory. Personal experience bears out this hypothesis for it was found that if the morning feeding was delayed by one hour the peak of the mitotic curve occurred at 3 p.m. instead of 2 p.m. Further, there appears to be an inverse relation between bodily activity and mitotic activity (Bullough, 1948*b*) for a high mitotic activity is associated with sleep and rest and a low mitotic activity with waking and exercise.

Effect of adrenaline and cortisone on mitotic cycle

Green and Ghadially (1951) first demonstrated the powerful antimitotic activity shown by adrenaline and cortisone on mouse epidermis. In their preliminary report only counts at the peak period (2 p.m.) were made, but a prolonged depressant effect is clearly shown in the present work. Later, Bullough (1952*a*) obtained essentially similar results. He found that adrenaline had a powerful antimitotic action both *in vivo* and *in vitro* and that the same is true of cortisone. He also studied the effect of stress, induced by overcrowding adult male mice, on adrenal size and epidermal mitotic activity. After 3 weeks the size, expressed as the maximal sectional area, of the adrenal medulla of the crowded mice increased by about 80 per cent while that of the cortex increased by about 30 per cent. Simultaneously the epidermal mitotic rate fell by about 60 per cent. Bullough (1948*b*) has also shown that exercise depresses mitotic activity, and the results reported in this paper confirm this finding. This effect may largely be due to the release of adrenaline into the circulation.

In our cortisone experiments, control animals were injected with saline, as the aqueous suspending fluid was not available at that time. However, Bullough (1952*a*) found that this fluid had no mitotic depressant effect in the intact animal although it had such an effect in high concentrations *in vitro*. Hence the use of saline instead of the suspending agent does not affect our conclusions.

It appears that both adrenaline and cortisone exert their antimitotic effect in the antephase, *i.e.*, these compounds seem to have no effect on any mitoses already in progress. There is no piling-up of cells in one phase of mitosis such as is induced in metaphase by colchicine. The action of these hormones more resembles that of iodoacetate, cyanide and dinitrophenol which all inhibit mitosis in the antephase (Bullough and Johnson, 1951*a*, *b* ; Bullough, 1952*b*). Hence Bullough suggests that their mode of action may be similar, for all these substances interfere with some stage of carbohydrate metabolism, as do adrenaline and cortisone. The effects of cortical hormones on carbohydrate metabolism are not clear, but

they seem to stimulate gluconeogenesis and decrease carbohydrate utilization (Long, 1949). However, Laws (1952) and Roberts, Florey and Joklik (1952) do not support Bullough's hypothesis, for they fail to find a positive correlation between available glucose and mitotic activity.

Ebert and Barclay (1952) have shown that cortisone produces prolonged vascular spasm in the rabbit ear, and the vasoconstrictor effects of adrenaline have been known for a long time. It is therefore tempting to suggest that both adrenaline and cortisone produce their antimitotic effect by a diminution of blood supply and hence metabolites to the epidermal cells. However, the fact that these drugs can exert their action both *in vitro* and *in vivo* (Bullough, 1952*a*) seems to point against such a simple explanation. Nor could vasoconstriction explain the diminution of mitotic activity in the lymph nodes, suggested by the work of Baker *et al.* (1951) and in the liver by Roberts *et al.* (1952) for it seems unlikely that simultaneous vasoconstriction occurs in every part of the vascular tree as a result of cortisone. It is well known, of course, that adrenaline constricts the vessels in the skin, but not those in the muscles and splanchnic area.

Adrenaline stimulates the hypophysis to liberate ACTH, thereby inducing the corticoid secretion from the adrenal glands (Vogt, 1945; McDermott, Fry, Brobeck and Long, 1950; White, 1947). Hence at least part of the effect of adrenaline on mitotic activity may be an indirect one, mediated *viâ* the pituitary-adrenocortical system. That this is not the full answer is suggested by the fact that adrenaline can exert its effect both *in vitro* and *in vivo* (Bullough, 1952*a*).

The depression of mitotic activity first demonstrated by Green and Ghadially (1951) suggests a possible explanation for some of the known effects of cortisone. Thus several studies have indicated that the glucocorticoid hormones interfere with wound healing (Baker and Whitaker, 1950; Bangham, 1951; Billingham *et al.*, 1951). They caused depressed cellular proliferation, delayed healing, defective granulation tissue formation, etc. All this could be explained by a depressed mitotic activity in the various tissues in the region (Green, 1950). It has also been shown that ACTH suppresses the growth of epidermis, sebaceous glands and hair (Baker *et al.*, 1948; Baker and Whitaker, 1950) and that cortisone reduces the size of sebaceous glands (Castor and Baker, 1950). Such observations would accord with its antimitotic activity.

However, it is unlikely that cortisone depresses mitotic activity in every tissue in every species, and it would be unwise to generalize on the basis of a few observations. The work of Roberts *et al.* (1952) suggests that while cortisone depresses mitotic activity in the regenerating liver it does not do so in the intestinal epithelium of the mouse. Mitoses were also seen by these authors in lymphoid follicles in mice receiving cortisone, but they do not clearly state whether mitotic depression occurred in this tissue or not. Baker *et al.* (1951) have, however, suggested that mitotic depression does occur in lymphoid tissues. It must, however, be noted that the findings of Baker *et al.* (1951) and of Roberts *et al.* (1952) are based on subjective impressions about the number of mitoses seen in their preparations, and not on quantitative methods as have been employed by us and by Bullough.

Effect of adrenalectomy, sham-operation and exercise on mitotic cycle

The effect of adrenalectomy on the mitotic cycle was investigated, for it seemed possible that removal of the source of these mitotic depressant hormones might

lead to increased mitotic activity. The difficulty here was that both the anaesthetic and the operative trauma might themselves stimulate adrenal hormone output. This would only be a temporary effect, but also unknown compensating mechanisms might come into action for a time and tend to mask the true picture.

It was observed early that the pattern of mitotic activity was easily upset by any condition causing stress such as rough handling, chilling, struggling, etc. Stress-producing stimuli, such as over-crowding (Bullough, 1952*a*), and exercise (Bullough, 1948*b*), depress mitotic activity, and other examples are presented in this work.

With this point in mind, we can explain why the mitotic activity in the sham-operated group of animals is severely depressed 48 hr. after adrenalectomy while in adrenalectomized animals the peak of the curve is within normal limits and the rest of the curve is slightly higher than in normal mice. It appears that the stress caused by the sham-operation is responsible for the depressed mitotic activity. On the other hand, in the adrenalectomized group, mitosis continues and it can be concluded that the adrenal is the source of the mitotic depressant hormones. There is, in fact, evidence of some stimulation of mitotic activity due to the absence of the adrenals.

Eighteen days after operation both the adrenalectomized and sham-operated groups showed a more or less normal mitotic cycle. This is, of course, understandable in the sham-operated animals, but a heightened mitotic activity in the adrenalectomized group might be expected. We have no explanation of why this was not so, beyond the possibility of compensating mechanisms coming into play. That these adrenalectomized animals were not capable of normal hormone production is revealed by their response to exercise. Exercise depressed the mitotic activity of both intact controls and sham-operated animals, whereas in the adrenalectomized group it was not affected. These results strongly support the hypothesis that at least one of the mechanisms by which a stress-producing stimulus leads to depression of mitotic activity is by the liberation of adrenal hormones.

However, many systemic mitotic depressants act directly for they are active in tissue culture. Thus for instance, Green and Ghadially (1951) found that adenosine depresses epidermal mitotic activity not only in the intact but also in the adrenalectomized animal. Stoner and Green (1950) showed that adenosine did not stimulate adrenal cortical activity and Hughes (personal communication) has also observed in tissue culture experiments with chick-embryo fibroblasts that adenosine depresses mitotic activity. Nevertheless, the present work does establish that in whole animals, control of epidermal mitotic activity is intimately related to the adrenal gland.

SUMMARY

The mitotic cycle of epidermal cells has been studied in the ear of Wistar rats, CBA mice and WLL mice maintained under standard environmental conditions. Quantitatively and qualitatively the results obtained were very similar in all these animals. Mitotic activity was low at 10 a.m. and high at 2 p.m., under the conditions of the test.

Adrenaline and cortisone given by subcutaneous injection both severely depressed mitotic activity.

Some evidence of enhanced mitotic activity at other than the peak period was obtained in mice 48 hours after total adrenalectomy, but sham-operated controls showed a marked depression of mitotic activity.

Mitotic studies made on the ear epidermis 18 days after operation revealed a more or less normal mitotic cycle in both adrenalectomized and sham-operated animals.

Exercise caused no depression of mitotic activity in adrenalectomized animals but produced a powerful depression in controls with intact adrenals.

It is concluded that at least one of the mechanisms by which a stress-producing stimulus leads to a depression of mitotic activity is by the liberation of adrenal hormones.

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