CERTAIN DATA RELATING TO THE MECHANISM OF SECRETION AND ACTION OF ADRENOCORTICOTROPHIC HORMONE (ACTH) AND OF CORTISONE IN EXPERIMENTAL INFLAMMATORY CONDITIONS

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Numerous papers have appeared in recent years, reporting the successful use of hypophysial extracts, adrenocorticotrophic hormone (ACTH), or cortisone in the treatment of inflammatory processes, both in experimental animals and in patients [1, 4, 20, 22]. The mechanism of action of ACTH and of corticosteroids has not yet, however, been elucidated. The antiedema effect of ACTH and cortisone is ascribed to their lowering of the permeability of the walls of the blood vessels, due to changes in the function of the sympathetic nervous system [15], and to lowering of the levels of blood hyaluronidase and histamine [16]. Increase in the content of plasma proteins due to treatment with ACTH has been reported [2], and this may also contribute to abolition of edema. There is no unanimity of opinion as to the mechanism of enhancement of the adrenocorticotrophic function of the hypophysis. According to some workers, the secretion of ACTH in response to trauma is regulated by both reflex and humoral pathways [3, 7, 14, 21]; in this relation, N. V. Mikhailova [10], I. I. Rusetskii [11], and others stress the dominating role of the nervous system. On the other hand, certain authors [17, 19, 22] believe that regulation of the adrenocorticotrophic function of the hypophysis can be achieved by humoral factors alone, without the participation of the nervous system.

The contradictory nature of the published material cited above led us to undertake the present research. Its object was to elucidate the effects of nervous and humoral factors on secretion of ACTH by rabbits suffering from inflammation due to thermal injury, and also to investigate the effect of ACTH on the course of inflammatory conditions of the ear under conditions of intact innervation and of desympathization.

EXPERIMENTAL METHOD

Our animal material consisted of grey rabbits, weighing from 2 to 2.5 kg. Thermal injury was inflicted by dipping an ear in water at 80° for 3 seconds. We performed 5 series of experiments: 1st – control series; 2nd series – scalding after division of the sensory nerves (Auricularis major et posterior); 3rd series – scalding after exclusion of the sympathetic nerve supply of the ear; 4th series – treatment of the scald with ACTH (intramuscular injection of 1 unit per kg every 6 hours for 7-10 days); 5th series – ACTH treatment (as in the 4th series) of scalds of the desympathized ear.

The following observations were made over a period of 6-15 days after infliction of the injury: size of swelling, temperature of the site of inflammation and rectum eosinophile count [by N. V. Mikhailova's method [10]] and leucocyte count of venous blood taken from the unaffected ear, and phagocytic activity of the leucocytes towards killed Staphylococcus aureus. The eosinophile counts were made $1\frac{1}{2}$ hours after taking the blood samples up into the mixing pipet. Eosinopenia was regarded as indicating activation of the function of the hypophysis – adrenal cortex system [22 and others]. We also recorded the time required for formation of a dry scab, and the duration of the inflammatory process (to when the scab was shed). The extent of necrosis was expressed as percentage of necrotic surface, in relation to the total surface of the scald. After detachment of the scab,

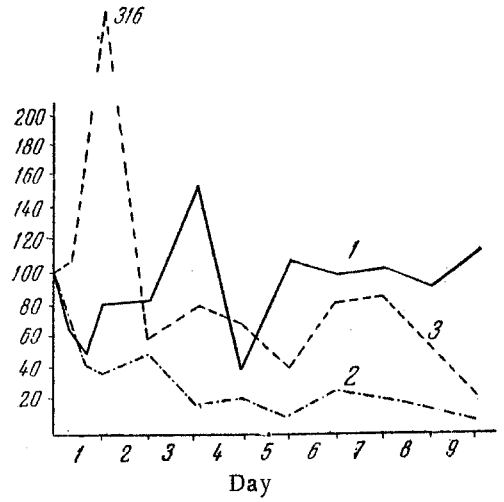

Fig. 1. Variations in the percentage content of eosinophiles in the peripheral blood of rabbits after infliction of thermal injury (mean values for each series)
1) Changes in percentage content of eosinophiles in the control experiments; 2) the same, for desympathized animals; 3) the same, for experiments involving deafferentation; ordinates – percentage content of eosinophiles in peripheral blood (the initial level is taken as 100); abscissae – days after infliction of injury.

we assessed the resistance of the animals to fatal scalding. This was done by pouring 1 liter of water at 100° on each of the hind legs, and then recording survival time.

EXPERIMENTAL RESULTS

Triggering mechanism for enhancement of adrenocorticotrophic function of the hypophysis in inflammation following thermal injury. As was mentioned above, a number of authors have shown that enhancement of the adrenocorticotrophic function of the hypophysis leads to a fall in the eosinophile count of the peripheral blood. Numerous authors [10, and others] take as evidence of increased secretion of cortisone the fall in the eosinophile count of peripheral blood, rather than their percentage content. In our opinion this procedure may be misleading, inasmuch as it is known that a number of pathological processes (including burns) are inaugurated by release into the circulation of blood having a high content of formed elements, from body depots. As a result, the eosinophile count rises, without much change in their percentage content. It is obvious that under such conditions it is possible to encounter an increase in the eosinophile count at the onset of a pathological state, instead of an eosinopenia, even although the level of corticoadrenal function has been raised; in such cases only a fall in percentage eosinophile content would reveal an early reaction to thermal trauma. For this reason we based our analysis of changes in adrenocorticotrophic function of the hypophysis on changes in the percentage content of eosinophiles in peripheral blood (Fig. 1).

We found in our control experiments that enhancement of corticoadrenal function took place in two episodes, as shown by eosinopenic episodes on the first and on some subsequent day of the experiments. In most of the experiments involving deafferentation we found an increase in the percentage content of eosinophiles, rather than an eosinopenia. This phase was then succeeded by a more pronounced eosinopenia than in the control experiments. At the same time, we observed more extensive necrosis in this series than in the controls, the necrotic area being 8% greater. Eosinopenia also proceeded biphasically in the experiments involving desympathization of the ear, the effects here being more pronounced than in the control series. The correlation between the degree of eosinopenia and the extent of the necrotic area was clearly evinced as the inflammatory process developed. In the experiments of this series the inflammatory process was associated with considerable edema (Fig. 2), and proceeded to necrosis of 94% of the scalded area (viz., 11% more than in the control series). Data relating to the duration of the inflammatory process, to the dimensions of the necrotic area, and to incidence of suppuration are recorded in Table 1.

Thus the initial rise in adrenocorticotrophic function of the hypophysis seen after scalding of intact and desympathized ears was absent when the afferent nerves of the ear had been divided. In the second phase, the degree of eosinopenia varies parallel with the intensity of the inflammatory process, and with the extent of the area of necrosis, irrespective of whether the afferent nerves of the affected region were divided or intact. These findings provide evidence, in our opinion, that in the first phase the mechanism triggering off enhancement of adrenocorticotrophic function of the hypophysis is preponderatingly of a reflex nature (stimulation of receptors in the inflammatory site), whereas in the second phase it is chiefly of a humoral nature (passage of breakdown products from the site of injury to the blood stream).*

* Analogous findings were obtained by us in recent experiments on white rats.

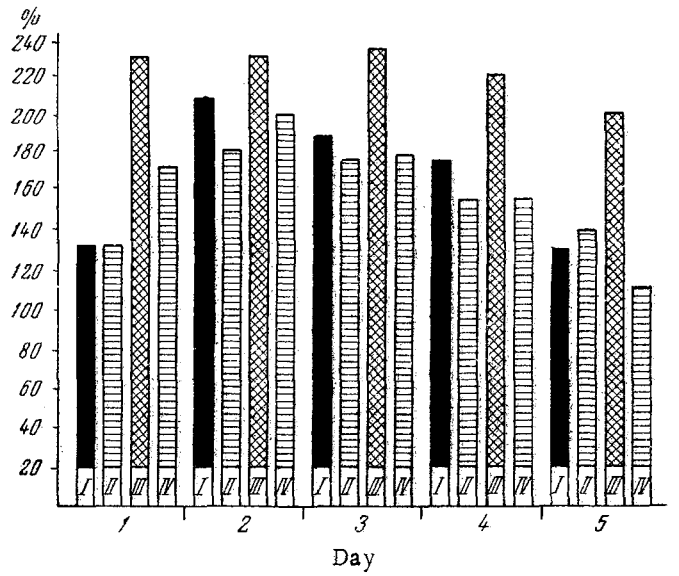

Fig. 2. Changes in the volume of rabbits' ears after scalding (mean results for each series of animals).
I) control series; II) experiments in which the animals received treatment for the burns; III) experiments in which the sympathetic nerve supply to the ear had been cut before scalding; IV) the same, with ACTH treatment; ordinates – volume of the affected ear, as percent of the initial volume, taken as 100%; abscissae – days after infliction of injury.

Effect of ACTH on the course of the inflammatory process. We found that the rise in temperature of the inflamed region was greater in animals treated with ACTH than in the control series: the temperature of the scalded ear exceeded that of the intact one by 1.8 – 4.4°, as compared with a difference of 1.1 – 2.9° found in the control series. This is evidence of the smaller disturbances in the local circulation [9], and confirms published reports of the intensification of metabolic processes caused by ACTH and cortisone [3, 18]. The ear was less swollen, from the first day of treatment (see Fig. 2). Similar findings have been reported by some authors [for example, [6, 15]]. Alleviation of the febrile condition reported by other authors to result from ACTH treatment [12] was not observed in our experiments. We found a greater leucocytosis in this series throughout the period of observation than in the control series. The phagocytary activity of the leucocytes rose steadily, although slightly (Table 2), which is in accordance with the findings of other workers [6].

TABLE 1

Duration of the Inflammatory Process, Extent of the Necrotic Area, and Frequency of Suppurative Complications of Scalds of Rabbits' Ears

Series	Day on which a dry scab formed	Duration of inflammatory process, in days	Extent of the necrotic area as % of total no. of expts.	Frequency of suppurative complications as % of the total no. of expts.
	2	3	4	5
1-	9-	22	83	40
2-	8-	23	91	20
3-	12-	32	94	90
4-	7-	24	79	—
5-	6-	21	96	—

Note: The figures under columns 2, 3 and 4 are mean values for a given series.

The fall in the percentage content of eosinophiles was more pronounced than in the control experiments. We found, however, that there was a transient rise in the eosinophile content of the peripheral blood on the 2nd-3rd day, in all the experiments. A dry scab was formed 2 days earlier, but it was shed 2 days later, than in the control series (see Table 1). The period of healing under the scab was thus prolonged by 4 days, which is evidence of retardation of proliferative processes due to the action of ACTH. In no case did suppuration supervene.

We thus found that ACTH treatment reduced the swelling and circulatory disturbances at the site of inflammation as compared with the control animals; the

TABLE 2
Phagocytic Activity of Rabbit Leucocytes after Thermal Injury.

Series	Day of experiment			
	1	2	4	6
1	2.9	2.4	1.6	2.7
4	1.6	1.7	1.8	1.7

Note: The initial level of phagocytic activity of the leucocytes is taken as unity. The figures given are the mean values per series.

extent of necrosis was also smaller. An unfavorable aspect of ACTH treatment is its depressing effect on the phagocytic activity of leucocytes, as well as its retarding effect on proliferative processes. Apart from these drawbacks, ACTH treatment had no effect on the febrile symptoms, and it caused an increase in the leucocyte count, as compared with the control series, a transient eosinophilia appearing on the 2nd-3rd days. These side-effects of ACTH treatment may have been due to the high content of protein contaminants in our preparations; this possibility, however, requires experimental confirmation.

Dependence of the therapeutic effect of ACTH on the sympathetic innervation of the site of inflammation. In the 5th series of experiments we applied ACTH to the treatment of burns of desympathized ears. We found that the temperature of the inflamed parts of the ear exceeded that found without treatment (3rd series): the difference between the temperature of the scalded and the intact ears varied from 2.6 to 4.5°, as compared with a range of 1.3-3.5° for the animals of the 3rd series. The ears were less swollen than for the animals of the 3rd series, or even of the control series, although more so than for the animals of the 4th series, in which ACTH treatment was given to rabbits with intact innervation of the ear (see Fig. 2). These findings are evidence that ACTH has a suppressive effect on edema of tissues deprived of their sympathetic supply. This result is not in accordance with N. P. Smirnov's interesting findings [15] that the sympathetic nervous system is involved in the lowering of capillary permeability taking place after administration of ACTH. It is possible that desympathized structures become more sensitive to the action of hormones, and not only to the corresponding mediators, as was shown by W. Cannon and A. Rosenblueth [5].

Suppuration was not encountered in this series, although in the 3rd series (untreated scald of a desympathized ear) it occurred in 90% of the cases. Finally, ACTH treatment of scalded desympathized tissues greatly shortened the duration of the inflammatory process, although the extent of the necrotic area was no smaller than in the experiments of the 3rd series. This finding points to the importance of the trophic function of the nervous system for the resolution of the inflammatory process; when this function is impaired treatment designed to alleviate secondary alterative changes (improvement of local circulation mediated by ACTH) are not effective in restricting the extent of the necrotic area.

Some data on the significance of the hypophysial-corticoadrenal system in the elaboration of immunity to the effects of burns. The possibility of developing immunity to the effects of burns has been demonstrated by the work of S. V. Skurkovich [13] and others. The results of our experiments confirm this finding. Whereas scalding of the hind legs of previously untreated animals caused their death in every case, with a mean survival time of 58 hours, of 8 rabbits which had recovered from scalding of an ear, without being given ACTH, two survived, and the mean survival time of the remaining 6 was 71 hours. Even greater resistance to lethal scalding was found for animals which had been treated with ACTH after scalding one ear; of 10 rabbits with scalded hind legs three survived, and the mean survival time of the remaining seven was 81 hours.

Our findings testify to the active participation of the hypophysial-corticoadrenal system in raising the resistance of animals to lethal scalding after recovery from minor burns.

SUMMARY

The course of the inflammatory process following scalding of one ear of rabbits, with and without previous deafferentation and desympathization of the ear, and with and without ACTH treatment, has been studied. The response of the adrenocorticotrophic function of the hypophysis proceeds biphasically, being triggered off by reflex action in the first phase, and by humoral action in the second. The temperature of the inflamed area is raised during ACTH therapy, while the swelling and the extent of the necrotic area are diminished and the incidence of suppuration is greatly reduced. With previous severance of sympathetic connections, ACTH treatment is also associated with higher temperature of the scalded region, edema is reduced, and the duration of the inflammatory process greatly shortened. The amount of necrosis is not, however, decreased. Rabbits which

have recovered from a minor burn are subsequently more resistant to major thermal injuries. The hypophysial-corticoadrenal system is actively involved in such raising of resistivity to thermal injury.

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