THE PARADOXICAL ACTION OF LARGE DOSES OF CORTISONE IN INFECTIVE AND ASEPTIC INFLAMMATION

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Cortisone is one of the most active anti-inflammatory preparations in clinical use at the present time. It is mainly given in hyperergic inflammation, for example in rheumatism. The administration of cortisone to patients with infectious conditions was considered to be contraindicated on the grounds that the drug lowered the resistance of the body. During recent years this view has been examined more closely and partially revised in connection with reports of the beneficial effects of small doses of cortisone in the treatment of a number of infectious diseases [1, 3, 6, etc.], and that the action of the drug depends on its dosage. The anti-inflammatory action of cortisone has been studied mainly in experimental aseptic inflammation; for this reason it is not certain to what extent the relationships found under these conditions are applicable to the more complex, infective inflammation. It is also uncertain to what general extent and with what consequences for the body the inflammatory reaction may be suppressed by the use of cortisone. In order to investigate these problems we studied the action of various doses of cortisone on inflammatory foci produced in white mice by subcutaneous inoculation of a culture of Friedländer's bacillus and of a solution of agar-agar.

EXPERIMENTAL METHOD

Mice weighing 14-16 g were inoculated subcutaneously in the right upper quadrant of the abdomen with a 20-hour culture of Bacillus pneumoniae suspended in physiological saline in proportion of 20-40 organisms to 0.04 ml of fluid. The virulence of the culture was maintained by daily passage through animals.

In another series of experiments, 0.04 ml of 0.008% solution of agar-agar in a 0.85% solution of NaCl was injected in the same region. 24 hours after injection of the culture of the microorganisms, and 4 hours after injection of the agar, the animals were killed and from the region of the injection were excised fragments of loose connective tissue, which were spread out on the surface of glass slides, fixed for 40-60 minutes in Shabadash No. 1 fixing agent* and then stained by Romanowsky's method. The number of the different cells in an area of 5.678 mm^2 of the stained films was counted by means of an ocular micrometer under the microscope with a magnification of 7 × 90. The areas of tissue selected for counting were either those with an accumulation of microorganisms (staining well by Romanowsky's method) or those with the most marked accumulation of polymorphonuclear leucocytes. The remaining fields of vision were selected from those in the immediate vicinity of the original.

The index of intensity of inflammation which we used was the ratio between the number of granulocytes and the total number of cells in the areas examined. In addition to this, in the experiments on infective inflammation, the number of organisms in the preparation was determined. The number of organisms in individual cases was enormous and could not be counted accurately.

We therefore counted their numbers within the following ranges: from 1 to 10, from 10 to 100 to 1000 and over 1000 in the preparation.

* Spiritus vini 100.0; $CuCO_3$ 1.8; $CaCO_3$ 0.9; neutral formalin 40% 10.0.

Cortisone acetate was injected under the skin of the dorsum in the form of a suspension in 0.85% NaCl solution. The last (or only) injection of cortisone was given 24 hours before giving the agar-agar or culture of microorganisms.

TABLE 1

Proportion of Leucocytes to Total Number of Cells in Films from Healthy Animals in Different Counts

Count	1	2	3
Proportion, %	5.58 ± 0.8	6.325 ± 1.1	5.83 ± 0.38

TABLE 2

The Effect of Cortisone on the Number of Leucocytes in an Inflammatory Focus during Infective Inflammation

Group	Dose of cortisone, mg/kg	Number of animals in group	Leucocyte content as a % of the control group	Index of reliability of the difference t	
				of the control group	of group No. 3
1	1.3 daily for 3 days	7	58.6	2.42	
2	4 once only	7	74.2	1.675	–
3	4 daily for 3 days	14	51.05	3.25	–
4	20 once only	7	85.0	0.77	1.83
5	20 daily for 3 days	21	117.1	0.961	3.73

Note: Leucocyte content of the control group of films – 24.8 ± 2.54% of the total number of cells, where n (number of animals) = 28.

TABLE 3

Number of Microorganisms in Films of the Connective Tissue from the Region of the Inflammation after Administration of Various Doses of Cortisone to the Animals

Dose of cortisone, mg/kg	Number of animals in group	Number of microorganisms in the preparation			
		from 0 to 10	from 10 to 100	from 100 to 1000	over 1000
		number of animals			
None given (control)	28	20	3	3	2
1.3 daily for 3 days	7	5	0	1	1
4 once only	7	6	0	0	1
4 daily for 3 days	14	10	1	1	2
20 once only	7	3	2	2	0
20 daily for 3 days	21	7	4	4	6

TABLE 4

The Effect of Cortisone on the Number of Leucocytes in an Inflammatory Focus during Aseptic Inflammation

Group	Dose of cortisone, mg/ kg	Number of animals in the group	Number of leucocytes as % of control group	Index of significance of the difference t	
				of control group	of group No. 4
1	1.3 once only	22	87.0	1.973	–
2	1.3 daily for 3 days	9	73.0	2.94	–
3	4 once only	12	75.1	2.84	–
4	4 daily for 3 days	5	68.0	3.78	–
5	20 once only	10	113.9	1.635	2.68
6	20 daily for 3 days	15	82.72	1.925	1.3

Note: The number of leucocytes in the control group of films was 58.15 ± 2.8 % of the total number of cells, wher n = 34).

EXPERIMENTAL RESULTS

The reliability of the method of counting the number of cells in the films was tested as follows. Films of connective tissue were taken from 8 healthy mice by the method described above. Independent counts of the different cells were carried out on a given area of these films by three persons. It was found that the variations in the total number of cells in various areas of the films and in different animals were considerable, but the percentage of leucocytes in these areas was quite constant, as can be seen from Table 1.

The mean difference between the indices obtained by different persons in counts of the same preparations was: between the 1st and 2nd – 0.625, between the 1st and 3rd – 1.975 and between the 2nd and 3rd – 2.156.

The probability of the independence of the indices obtained in the 1st and 2nd counts, calculated by the Student distribution, was p = 0.558, and that of the 1st and 3rd counts, p = 0.927.

It could thus be considered that the number of leucocytes in the connective tissue films from normal animals was quite constant, and the individual characteristics of the experimenter in the selection of a place for the counting and in classifying the cells were of no essential importance.

The changes in the number of leucocytes in the inoculated mice are shown in Table 2. When this Table is examined it must be borne in mind that after subcutaneous injection of B. pneumoniae, irrespective of the virulence and number of the organisms injected, death of the animals did not begin to take place earlier than 30 hours after infection, and so none of the experimental animals died spontaneously.

As may be seen from Table 2, the single or repeated injection of cortisone acetate in a dose of 4-12 mg/ kg led to an obvious and statistically significant decrease in the infiltration of the focus of infection with leucocytes. Increasing the dose of cortisone to 20-60 mg/ kg not only did not cause any further decrease in infiltration but, on the contrary, this was even enhanced.

In order to explain this feature it was essential to take into account the characteristics of the change in the number of microorganisms in the focus. As may be seen in Table 3, when large doses of cortisone were given there was a considerable increase in the number of microorganisms developing from an initial number of several tens of bacilli injected. It might be assumed that an increase in the number of microorganisms in the region of infection intensified the degree ofstimulation. On the other hand, this increase in number was itself, evidently, the consequence of the reduced activity of factors of defense, which in ordinary conditions restrain the proliferation of microorganisms injected into the tissues.

In the aseptic inflammation experiments the main features of the relationship between the degree of infiltration and the dose of cortisone were the same as in the experiments using the culture of B. pneumoniae (Table

4). The anti-inflammatory effect was gradually intensified as the dose of cortisone was raised from 1.3 to 12 mg/ kg, but further increase of the dose of cortisone led to perceptible weakening of the degree of suppression of infiltration, the differences between the actions of doses of 12 and 20 mg/ kg being sufficiently significant.

The relationship thus observed was not, evidently, a special peculiarity of the anti-inflammatory properties of cortisone, but was due to peculiarities of the reaction of the body to this form of experimental inflammation. In any case, in joint observations with N. A. Demidova, using the same method, on the anti-inflammatory action of diazolin, obvious suppression of infiltration was observed in response to the administration of 10 mg/ kg, whereas this effect was completely absent when the drug was given in a dose of 25 mg/ kg.

Cortisone has a very complex and widespread action on the various systems of the body, associated with the development of defensive reactions. The mechanism of its action on inflammation is not completely clear. Dougherty and Schneebeli [2] observed accumulation of hydrocortisone in the fibroblasts, and pointed out the characteristic rounding of these cells in animals which had been given injections of cortisone and hydrocortisone, and the high resistance of fibroblasts of a spheroidal shape to the action of substances tending to destroy them. These authors were inclined to connect the anti-inflammatory properties of corticosteroids with this effect on the fibroblasts. On the other hand, the findings of Opsahl et al. [4], Winter and Flataker [8], and Seifter et al. [5], and our own observations showed that the functional properties of the basic substance were essentially modified by the action of cortisone. In parallel experiments we were able to show that a fall in the permeability of the basic substance to the spread of dyes and of bacteria could be observed after administration of small doses of cortisone, having an anti-inflammatory action. This effect on the basic substance ceased to be found in response to injection of doses of 20 mg/ kg and above. Thus the change from small to larger doses of cortisone was associated with a number of changes in the functional condition of the tissues.

Spain and Molomut [7] observed increased tissue necrosis in areas of turpentine inflammation in animals receiving large doses of cortisone.

From the above it may be assumed that in response to cortisone the nonspecific resistance of the tissues to injurious agents is reduced, and this reduction becomes considerable in degree when the dose of cortisone is increased. This is clearly demonstrated by the sharp rise in the number of Friedländer bacilli in the corresponding films of connective tissue. The great degree of tissue injury evidently acts as an additional stimulus to intensify the inflammatory reaction, possibly through mechanisms not concerned with the effect of cortisone.

This hypothesis requires further proof and verification. On the basis of the facts described, however, it may be considered that in any case the degree of suppression of the inflammatory reaction by cortisone is confined between definite limits. The attempt to increase the anti-inflammatory action by giving more than the optimum dose is not only ineffective but also dangerous, on account of the fall in the activity of the humoral and tissue defensive factors.

The changes taking place in the local inflammatory focus are evidently the result of changes in the general reactivity of the body under the influence of cortisone, although the actual mechanisms by which cortisone affects the systemic reactions of the body have been studied very inadequately.

SUMMARY

The author studied the effect of various doses of cortisone on the aspetic (agar-agar) and infectious (B. pneumoniae) inflammation by the method of determination of the degree of the leucocytic activity of the friable connective tissue. The quantitative regularities governing the depression rate of the infiltration by different doses of cortisone were found to be the same in both types of inflammation. It gradually rises with the increase of the cortisone dose from 1.3 to 12.0 mg/ kg. By increasing the cortisone dose to 20 mg/ kg the infiltration becomes still greater. The apparent absence of any effect of large cortisone doses on the inflammation is due (in infectious cases) to a considerable increase in the number of microbes at the site of their introduction and points to the decline in the tissue factor activity of the body resistance.

LITERATURE CITED

[1] N. A. Shmelev, Problemy Tuberk., 3, 20-28 (1957).

[2] T. F. Dougherty and G. L. Schneebeli, Ann. New York Acad. Sc. , 61, 328-348 (1955).

[3] L. W. Kinsell and J. P. Jahn, Ann. New York Acad. Sc., 61, 397-407 (1955).

[4] Opsahl et al., Yale J. Biol. a. Med., 23, 399-406 (1951).

[5] J. Seifter, et al., Proc. Soc. Exp. Biol. a. Med., 75, 337-342 (1950).

[6] J. E. Smadel et al., Ann. Intern. Med., 34, 1-9 (1951).

[7] D. M. Spain and N. Molomut, Am. J. Clin. Path., 22, 944-947 (1952).

[8] C. A. Winter and L. Flataker, Proc. Soc. Exp. Biol. a Med., 79, 312-314 (1952).