EFFECTS OF HEPARIN AND ASBESTOS WITH CORTICOTROPHIN ON THE MUCOSAL MAST CELLS AND TISSUE EOSINOPHILS OF RAT STOMACH

By

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ABSTRACT

Intact rats were injected once with a suspension of asbestos and for 5 days with 2 IU of adrenocorticotrophin-zinc per day. 1.0 mg of heparin was injected intraperitoneally 9 times at 12-hourly intervals and the same amount of ACTH intramuscularly. The rats were decapitated 5 days after the injection of asbestos, 24 hours after the last ACTH injection and 3 hours after the last heparin injection. The mucosal mast cells and tissue eosinophils of the stomach were counted from the body mucosa and recorded per mm² of tissue.

Heparin caused no changes in either the mast cell count or tissue eosinophilia, nor did it bring about any changes in the degranulation of mucosal mast cells during the ACTH effect, or in the destruction of tissue eosinophilia. Asbestos peritonitis seemed to have a degranulating effect on mucosal mast cells and a destructive effect on tissue eosinophilia. It also appeared to increase the effect of ACTH on the mucosal cells.

It is suggested that glucocorticoids stimulated by ACTH exert such an immediate effect on the function of the cells of the mucosal lamina propria that the inhibitory effect of heparin and asbestos is counteracted.

Long-term corticotrophin (ACTH) therapy destroys almost completely the mast cells of the gastric body mucosa of rat; its effect is exerted via the adrenocortical hormones (Räsänen 1961). Blood eosinophils decrease in mice, the extent depending on the ACTH dosage (Speirs et al. 1953). Heparin in vitro (Muehrcke et al. 1952) and in vivo (Weissbecker & Schröter 1954; Braunsteiner et al. 1959) seems to inhibit the eosinopenic effect of cortisone and ACTH. Speirs

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(1955) observed that the inhibitory effect of heparin on ACTH eosinopenia was possibly caused by asbestos particles from a Seitz filter. The asbestos particles bring about a rapid and profuse eosinophilia in the abdominal cavity which persists for a long time.

The aim of the present work was to find out whether heparin inhibits the degranulating effect of ACTH on gastric mucosal mast cells and its destructive effect on tissue eosinophilia, and also whether asbestosis causes any changes in the mucosal mast cells and tissue eosinophilia of the stomach and alters their response to ACTH.

**METHOD**

The rats, the experimental conditions, preparation of the samples and the cell counts were as in the previous study. The group of rats receiving ACTH had 9 experimental animals, the other groups 10 animals each. ACTH was injected intramuscularly and the other substances intra-abdominally. The rats were decapitated 3 hours after the last heparin injection and 24 hours after the last ACTH injection. The asbestos injection was given 5 days before decapitation. The rats fasted for the last 16 hours.

The following injections were given:
1. Heparin (Pularin, Batch M 73990, Evans) 9 × 1.0 mg (= 125 USP) at 12-hourly intervals. The rats weighed 197.4 g (range 178–216);
2. Heparin as above + ACTH-zinc (Cortotrophin-Z, Organon) 4 × 2 IU at 24-hourly intervals. The mean weight of the rats was 199.8 g (range 168–224);
3. Asbestos in aqueous suspension 1.0 ml (= 1.0 mg of asbestos which was heated and sterilised). The mean weight of the rats was 210.4 g (range 188–228);
4. Asbestos as above + ACTH as in group 2. The ACTH therapy was started immediately after the asbestos injection. The mean weight of the rats was 182.8 g (range 164–214);
5. ACTH as in group 2. The mean weight of the rats was 171.8 g (range 178–224);
6. Controls, mean weight 179.2 g (range 168–212), which were given 6 × 0.5 ml of saline at 8-hourly intervals.

**RESULTS**

The undue toxic effects were observed during the experiment. The rats given asbestos had a slightly distended abdomen and grey coloured liquid formed intra-abdominally. The rats given heparin showed haematomas under the abdominal skin at the injection sites and elsewhere in the body, probably on account of the traumata.

The results of the cell count (expressed as the number of cells per mm² of tissue) are shown in Fig. 1.

ACTH caused a distinct degranulation of mast cells in the gastric body mucosa. The difference is statistically highly significant (P < 0.001). Heparin did not seem to affect the number of the mucosal mast cells nor definitely to inhibit their degranulation under the influence of ACTH.

In the rats given asbestos the mucosal mast cells showed some degree of
Fig. 1.
Mucosal mast cells and tissue eosinophils in rat gastric body mucosa after heparin, asbestos, and ACTH, and simultaneous ACTH and heparin or asbestos application, and in controls.

degranulation, but the difference was not significant. The degranulating effect of ACTH on mucosal mast cells increased in asbestosis, though this was not significant.

The decrease in tissue eosinophilia during ACTH therapy seemed clear ($P < 0.01$). Heparin did not seem to have any definite effect on ACTH-induced tissue eosinopenia. Asbestosis by itself and with ACTH slightly reduced tissue eosinophilia but this was not statistically significant.

From the equation $y = y_0 \cdot e^{-kt}$, in which $y$ = the number of cells/mm$^2$ ($y_0$ = the value of the controls), $t$ = the time, measured as intervals of time (= 24 hours), the following values are obtained for the degranulation and destruction coefficient of ACTH:

$$k = \frac{1}{4} \cdot \ln \frac{1080}{256} = 0.3599$$
$$k = \frac{1}{4} \cdot \ln \frac{2339}{1569} = 0.0900$$

**DISCUSSION**

Although heparin seems to inhibit the eosinopenic effect of ACTH in circulating blood (Weissbecker & Schrötter 1954; Braunsteiner et al. 1959), the present experiments have shown that its effect on mucosal mast cells and tissue eosinophilia is negligible. The effect of ACTH is probably transmitted to the lamina propria of the mucosa by glucocorticoids. Asbestos did not cause any increase in tissue eosinophils in the gastric lamina propria an effect which has been observed in the peritoneal fluid following the injection of asbestos fibres. In contrast, asbestosis increased the destruction of mast cells and eosinophils, probably because of the stress caused by the peritonitis.
Radioactive sulphate is rapidly bound with polysaccharides in the gastric mucosa (Kowalewski & Silbermann 1958) and enters the gastric juice depending on the intensity of the gastric stimulation. The polysaccharide of the gastric mucosa resembles heparin (Smith et al. 1952) and differs from the polysaccharide isolated from duodenal mucosa (Bianchini 1958). The microsome fraction of gastric mucosa contains a great deal of glucuronyl-transferring enzyme (Dutton & Stevenson 1959) the amount of which diminishes following radiation (Hartiala et al. 1958) and degranulation of mast cells occurs (Räsänen et al. under publication). It is possible that the granules of the mucosal mast cells form a glucuronide depot and enzymic centres in which the glucocorticoids are also conjugated, using up metachromatic material.

The relatively small quantity of ACTH used in the present investigation probably caused a condition in which degranulation of mast cells, obviously bound to their site, was greater than the destruction of the tissue eosinophils, which may be compensated in haematogenic way. When the exogenous glucocorticoid effect is sufficiently marked, as was the case in the previous investigation (Räsänen 1961), the relative destruction of tissue eosinophils becomes smaller than the relative degranulation of mast cells, presumably because the effect of the eosinopenia-producing metabolites overcomes the compensatory production of eosinophils by the organism.

Exogenous heparin introduces glucuronic acid into the organism. But heparin is rapidly secreted in a desulphurated form through the kidneys (Danishefsky & Eiber 1959). The effect of glucocorticoids which degranulate mucosal mast cells would also seem to be based on the presence of enzymes, probably in the mast cells. This chain of events is thus so closely tied up with the lamina propria and the enzymic economy of its cells, that a substitute material from outside has no significance.

REFERENCES


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