

## ORIGINAL ARTICLE

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# Myeloperoxidase activity in skin lesions

## II. Influence of alcohol and some medicines

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**Abstract** The measurement of myeloperoxidase activity was used to quantify the acute granulocyte reaction. Ethyl alcohol given intraperitoneally in rats as doses of 3, 4 and 5 g per kg body weight resulted in a clear decrease of the myeloperoxidase activity at the edges of incision wounds. When one-half of the LD 50 dose of chlorpromazine was given intraperitoneally a remarkable decrease in the myeloperoxidase activity was observed. Moderate decreases were obtained with one-half of the LD 50 dose of acetyl salicylic acid and Na-pentobarbital and with high therapeutic doses of hydrocortisone and indomethacin.

**Key words** Granulocyte reaction · Myeloperoxidase activity · Alcohol · Medicines

### Introduction

In forensic medicine it is often necessary to estimate the age of traumatic lesions. Although recently several new methods such as measurement of inflammatory cytokines or detection of different surface antigens of inflammatory cells [1–3] have been introduced for this purpose, the presence and the quantity of the inflammatory reaction is still often used. However, traumatic lesions have often been observed in persons who have consumed alcohol or medicines. From the literature it is known that at least some medicines e.g. corticosteroids and anti-inflammatory drugs [4, 5] are able to influence the inflammatory reaction as well as intoxication by hypnotics [6–8]. In 1938 experiments on rabbits showed that alcohol resulted in a retarded granulocyte emigration to the sites of intradermal pneumococcal infection [9]. Later the same effect has been demonstrated in skin abrasions of human subjects [10]. It has however, been reported that the dermal leuco-

cytosis was not influenced by high concentrations of alcohol in experimental wounds in guinea pigs although the activation of different structure bound enzymes was significantly delayed and decreased [11]. Because of these discrepancies in the literature the influence of alcohol on the quantity of the acute granulocyte reaction in experimental wounds was investigated in the present work by measuring myeloperoxidase activity, which is known to show good correlation with the number of granulocytes in tissue [12, 13]. In addition the influence of some medicines on the quantity of the granulocyte reaction in experimental wounds was investigated using the same method.

### Materials and methods

In the experiments male Sprague-Dawley rats were used (average weight 500–550 g). Each of experimental animals was first given a single intraperitoneal dose of 10% alcohol in physiological saline solution. The single alcohol dosages used were 3, 4, and 5 g per kilogram body weight of the animal. If an experiment lasted more than 12 h, each experimental animal was given a repeat dose of alcohol identical to the initial dose received. An incision wound about 5 cm long which perforated the skin was made on the dorsum of the experimental and control animals 30 min after the injection of alcohol. Six rats with each dosage of alcohol or without alcohol were then sacrificed at 4, 12 and 24 h after wounding. Zones of about 1 mm in thickness were taken from the edges of the wounds for the myeloperoxidase activity determinations.

In the second series of experiments the medicines studied were given intraperitoneally 30 min before the infliction of the wound on the dorsum of the rats. If the experiment lasted longer than 12 h the same dose of the medicine was repeated. High therapeutic doses were used for hydrocortisone (Solu-Cortef, Upjohn) and indomethacin (Confortid, Dumex) the exact values being 15 mg per kg and 10 mg per kg respectively. For the other medicines half of the LD 50 for intraperitoneal application in rats [14] was used. The exact values were 250 mg per kg for acetylsalicylic acid, 36 mg per kg for amitriptyline, 37 mg per kg for chlorpromazine and 37.5 mg per kg for sodiumpentobarbital. Acetyl salicylic acid was made water soluble by adding sufficient sodium hydroxide to a suspension in 0.1 M phosphate buffer to make an approximately neutral solution. For each medicine tested or without medicine six rats were sacrificed at 4, 12 and 24 h after wounding. The specimens for myeloperoxidase determination were taken as described previously. The myeloperoxidase determinations were done as previously described [15]. The activities were expressed as units per g dry

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weight (U/g dw) The rats were anesthetized with ether throughout the experimental procedures. The experiments were approved by the local committee for animal experiments. The statistical analysis of the results was performed using Student's *t*-test. In the figures *n* = 6 indicates that in each curve at each time point presented, the mean represents observations from 6 experimental animals.

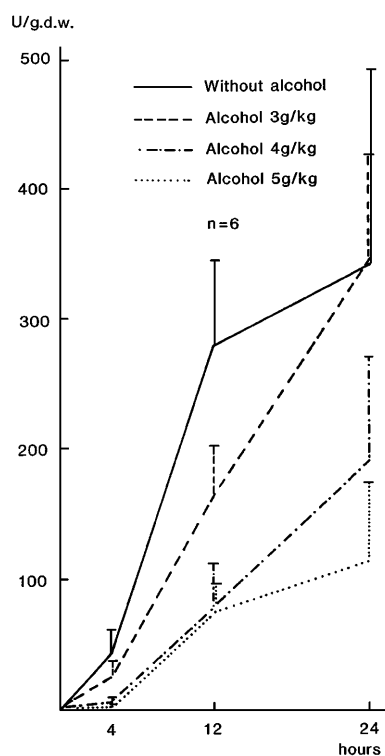
## Results

### Alcohol

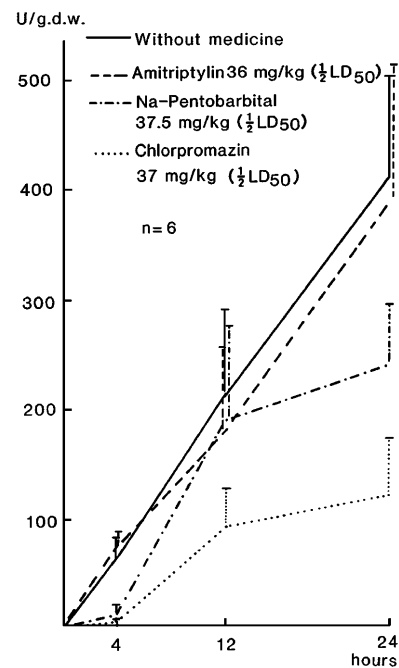
Myeloperoxidase activity in the wounds from rats which received 3 g per kg was approx 60% that found in controls ( $P < 0.01$ ) after 12 h, but after 24 h it had returned to the control level. In the wounds of the animals that had received doses of 4 and 5 g per kg the decrease of the activity was more remarkable and persistent ( $P < 0.001$ , Fig. 1).

### Medicines

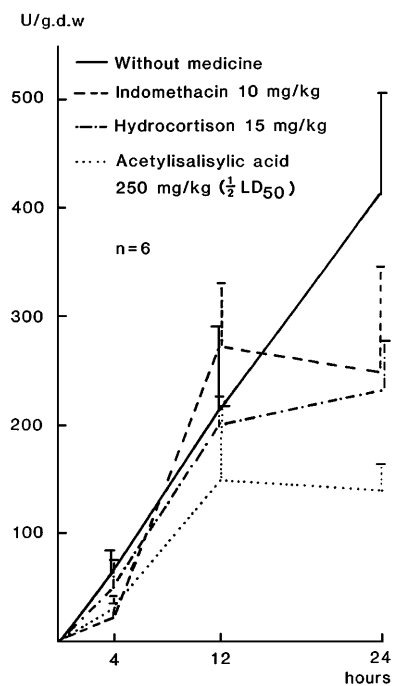
The most remarkable decrease of the myeloperoxidase activity was obtained for chlorpromazine ( $P < 0.001$ ). Acetylsalicylic acid, hydrocortisone and indomethacin produced a moderate decrease after 24 h and sodium pentobarbital after 4 h ( $P < 0.01$ ). Amitriptyline did not show any clear decrease from the control level (Figs. 2 and 3).



**Fig. 1** Retarding effect of different amounts of alcohol on the increase of the myeloperoxidase activity at the edges of the experimental wounds. Means and standard deviations are given



**Fig. 2** Effect of amitriptyline, Na-pentobarbital and chlorpromazine on the increase of the myeloperoxidase activity at the edges of experimental wounds compared with wounds in the control rats. Means and standard deviations are given



**Fig. 3** Effect of indomethacin, hydrocortisone and acetylsalicylic acid on the increase of the myeloperoxidase activity at the edges of experimental wounds compared with the wounds in the control rats. Means and standard deviations are given

## Discussion

### Alcohol

With increasing amounts of alcohol i.e. from 3 to 5 g per kg body weight, a progressive decrease of myeloperoxidase activity was observed at the edges of the wounds in the present experiments. Our results are in accordance with those of the other workers [9, 10, 16] who have shown that leucocyte migration from vessels to the site of an inflammation is remarkably inhibited by certain levels of alcohol. The highest amount of alcohol used in present experiments, 5 g/kg body weight given intravenously or intraperitoneally, has been described to be the LD 50 for rats [17]. In the present experiments however, this did not result in mortality of the rats. Acute ethanol intoxication is especially believed to reduce the adhesion of neutrophils to the endothelium of the vessels probably by the decreased expression of the neutrophil plasma membrane Mac-1 glycoprotein critical for correct neutrophil adhesion functioning [18, 19]. In humans, blood alcohol levels of 1.5‰ have been shown to inhibit the exudation of granulocytes into skin chambers after experimental abrasions [16].

### Medicines

The findings of the present work are in accordance with the earlier reports in the literature. Chlorpromazine has been shown to depress neutrophil chemotaxis and to induce neutropenia in vivo [20, 21]. It is well known that glucocorticoids are able to inhibit the migration of leukocytes into an inflamed area [4, 22, 23]. Impaired adherence of the granulocytes has also been demonstrated by in vivo administration of acetylsalicylic acid to human volunteers [18]. Of the non-steroid anti-inflammatory drugs, ibuprofen, naproxen and indomethacin have been shown to have inhibitory effects on leucocyte migration [4, 26, 27]. There are several reports in the literature that intoxication with hypnotics results in retardation and weakening of early cellular inflammation [6–8, 28]. Reports showing the depression of inflammatory reaction by amitriptyline were not found.

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