pH of Inflammatory Exudates in Granulocytopenic Rabbits[1] (37459)

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Recent studies from this laboratory showed an acid exudate pH in sterile, nonspecific inflammatory skin lesions of normal rabbits (1). Similar studies in rabbits with acute alloxan diabetes and blood acidosis suggested a correlation of the exudate pH with the intensity of the polymorphonuclear leukocyte response of the wounds (2). The present experiment, which like those previously reported employed standardized methods of producing lesions, collecting the exudates and precision pH measurements, investigated the effect of granulocytopenia on the exudate pH.

Materials and Methods. Animals. Male white New Zealand rabbits weighing from 1800 to 2400 g, housed in air-conditioned quarters, were fed standard chow and water *ad libitum.* Intravenous pentobarbital anesthesia was used at time of chamber placement and at sacrifice.

Granulocytopenia. A single intravenous injection of 2.5 mg/kg nitrogen mustard (HN_2) (Mustargen, Merck, Sharp and Dohme, Inc., West Point, PA) was given to all but 3 rabbits in the 24 hr group which received only 1.9 mg/kg HN_2. The drug was administered as previously described (3).

Hematology. Total and differential white blood cell counts, platelet counts and hematocrits were determined by standard methods.

Chamber. The chamber resembled that previously described (1) except for an enlarged sideport containing 1 cm of 16 gauge stainless steel tubing with 14 cm of polyvinyl tubing (id 0.066 in., od 0.095 in.) attached. The materials and procedures for producing the lesions, placement, attachment and filling of the chambers and for the collection of the exudates were as previously detailed (1, 2). Upon collection samples of the chamber contents (exudates) were cultured.

pH measurements. The determinations of blood and exudate pH were performed on unanesthetized animals in the same manner with the microelectrode of a pH meter as previously reported (2).

Histologic procedures. Fixation, staining and histologic study of the wounds were also done as before (1, 4). The overall intensity of the inflammatory cell response represented by the accumulation of inflammatory cells in the lesions as well as exudation, the process of white blood cell migration from the circulation into the site of injury, as gauged by the sticking of the cells to endothelium and their migration through the vessel wall were recorded semiquantitatively on a scale of 0 to 4+ as indices of the exudative inflammatory cell reaction.

Experiment. About 68 hr after HN_2 injection two skin wounds, one on each shaved flank, were produced and capped with chambers which were filled with buffer. The exudates were collected 24, 48 or 72 hr later and their pH measured. Immediately thereafter the animals were sacrificed and the wounds were excised for histologic study. Blood pH determinations were done before HN_2 injection as well as immediately before chamber placement and sacrifice. Total and differential white blood cell counts were made immediately before chamber placement and sacrifice and platelet counts and hematocrits were determined immediately before sacrifice. The data from normal rabbits of Expt B of a previous study (1) served as controls.

Results. The rabbits lost an average of 340 g during the experiment but showed no hemorrhagic manifestations and appeared healthy.

[1] Supported by U.S. Public Health Service Grant AM-04593-13 from the Department of Health, Education and Welfare.

TABLE I.[a]

Time (hr)	No. rabbits	Initial WBC (PMN)/mm³	Terminal WBC (PMN)/mm³	Terminal blood pH	No. chambers	Terminal exudate pH	Inflammatory response: Intensity	Inflammatory response: Exudation
24	6	1700 (156) 450–3350 (22–302)	1800 (215) 600– 5100 (15– 714)	7.382 7.327–7.435	9	7.330 7.272–7.379	0.67 0– ++	0 0
48	4	900 (52) 300–1100 (12– 88)	4600 (1690) 1400–10,500 (144–6200)	7.362 7.316–7.435	5	7.322 7.245–7.380	1.8 +–+++	1.8 0–+++
72	8	1200 (106) 900–1600 (36–336)	4700 (3200) 2300– 8200 (896–6070)	7.362 7.346–7.455	11	7.189 7.015–7.334	1.4 +– ++	0.64 0– +

[a] Averages (first line) and ranges (second line) of: white blood cell (WBC) and in parentheses polymorphonuclear leukocyte (PMN) counts, blood and exudate pH, overall intensity of inflammatory cell response and leukocyte sticking to endothelium and migration through vessel wall (exudation). Initial—at time of chamber placement. Terminal—at sacrifice.

At sacrifice platelet counts averaged 376,000/mm³ ranging from 15,000 to 780,000/mm³ (normal 837,000 ± 211,000/mm³) and the average hematocrit was 33% ranging from 25 to 41% (normal 38 ± 3.5%). Averages and ranges of total white blood cell and polymorphonuclear leukocyte counts at time of chamber placement and at sacrifice are shown in Table I (normal: WBC 8475 ± 2230/mm³, PMN 4245 ± 1275/mm³). At time of chamber placement a marked leukopenia was present. The polymorphonuclear leukocytes were more reduced in number than the other white blood cells. The lowest average values of total white blood cells and of polymorphonuclear leukocytes were found at time of chamber placement. Subsequent values showed gradual return toward normal.

All blood pH determinations remained within normal limits (pH 7.385 ± 0.041). The values at sacrifice are shown in Table I.

Exudate pH measurements were not obtained from every chamber since large fibrin clots, not encountered in the previous studies, sometimes interfered with the collection of the exudate. The difficulty was largely overcome by collection of the exudate through the enlarged sideport. The exudate pH values and the histologic grade of the overall intensity of the inflammatory cell response and the degree of white blood cell sticking to endothelium and their migration through vessel walls (exudation) are shown in Table I and in Fig. 1. The pH of the exudates at 24 and 48 hr was similar and high compared to the controls. At 72 hr the pH had decreased sharply but remained elevated compared to normal values. The inflammatory cell response at 24 hr consisted mostly of mononuclear cells and its overall intensity was minimal while at the corresponding time in the controls it was pronounced with a predominance of polymorphonuclear leukocytes. Some of the latter cells were present in addition to mononuclear cells at 48 and 72 hr but the overall intensity of the response was slight and distinctly less than in the controls. Leukocytic sticking and migration were absent at 24 hr and present at 48 hr and to a lesser degree at 72 hr while in the controls it was slight at 24 hr and absent at the latter

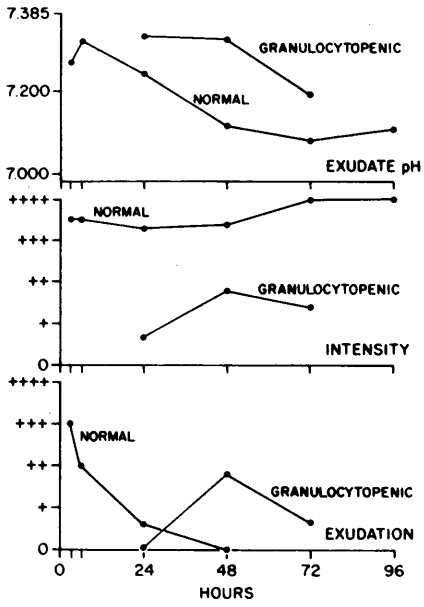

FIG. 1. Averages of: exudate pH (top) and histologic grade of inflammatory cell reaction gauged by overall intensity of inflammatory cell response (middle) and exudation, i.e., leukocyte sticking to endothelium and migration through vessel wall (bottom) in normal and granulocytopenic rabbits. Hours show time after chamber placement, which in the granulocytopenic animals was 68 hr after HN_2 administration.

times. Reparative processes appeared normal. All exudates were sterile.

Discussion. The changes following the administration of HN_2 to experimental animals have been studied extensively (5, 6). The single dose of HN_2 used in the present experiment produced a marked but transient leukopenia which affected the polymorphonuclear leukocytes to a more severe degree than the other blood cells. This finding which is in agreement with the observations of others (7, 8) indicates that granulocytopenia is the principal hematologic alteration in the experimental animals. The granulocytopenia was reflected in a distinctly reduced granulocytic infiltration of the wounds which while most marked at 24 hr was present also at 48 and 72 hr. In the 24 and 48 hr lesions the local acidosis found in the corresponding controls was absent and the exudate pH was similar to the blood pH which was normal. In contrast to the controls white blood cell margination and emigration were absent at 24 hr, showed a delayed onset with a peak at 48 hr and persisted to a lesser degree at 72 hr. The present data do not explain this curious finding. The granulocytic infiltration of the lesions at 48 and 72 hr which although less than in the corresponding controls was somewhat greater than that encountered at 24 hr appeared to correlate with a return toward normal of the total white blood cell and particularly the polymorphonuclear leukocyte counts. The larger number of granulocytes among the inflammatory cell infiltrate also appeared to correspond with a decrease of the exudate pH at 72 hr toward the values in the corresponding controls. At sacrifice the animals all of which had lost some weight, appeared to be recovering from the pharmacologic insult. The averages of the hematocrits and platelet counts were somewhat decreased but values within normal limits were present in each group of rabbits which showed marked granulocytopenia, impaired granulocytic infiltration in the lesions and no decreased exudate pH. The greatest effect on the exudate pH occurred at 4 and 5 days after HN_2 injection suggesting that destruction of circulating and maturing polymorphonuclear leukocytes rather than altered granulocyte function or immune mechanisms accounts for the observed changes.

In these short-term experiments a marked transient granulocytopenia resulted in a striking temporary reduction of granulocytes in the inflammatory response of the lesions. The absence of a local acidosis in lesions with severely impaired granulocytic response and conversely the presence of local acidosis in lesions with delayed granulocytic infiltration indicate that in acute inflammation the exudate pH depends in large measure upon the numbers of granulocytes at the site of injury.

Summary. The exudate pH from nonspecific acute inflammatory skin lesions of granulocytopenic rabbits was measured at various times and the inflammatory cell response in the corresponding lesions was studied histologically. In granulocytopenic animals acidosis was absent in early lesions with reduced granulocytic response but developed in later lesions showing delayed granulocyte infiltra-

tion. This study indicates that the local acidosis in acute inflammatory lesions depends largely upon the granulocytic infiltration at the site of injury.

The authors thank Miss Hillma Gheesling, Mrs. Julia Schuch and Mrs. Marjorie Lamb for competent technical assistance, and Mrs. Nancy Lambert for secretarial help.

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Received Feb. 14, 1973. P.S.E.B.M., 1973, Vol. 143.