The pH of Inflammatory Exudates in Acidotic Diabetic Rabbits[1] (36517)

G. M. HUTCHINS AND W. H. SHELDON

Department of Pathology, The Johns Hopkins University School of Medicine and Hospital, Baltimore, Maryland 21205

In a previous study (1) the exudate pH of the inflammatory response in nonspecific skin lesions of normal rabbits was determined over a period of 4 days. Acidosis was demonstrated at the site of injury while the blood pH remained unaltered. A correlation between the increased H-ion concentration in the wound and the attending inflammatory cell response was not apparent. The present experiment was designed to study the exudate pH and the development of the inflammatory cell response in nonspecific skin lesions of rabbits with acute alloxan diabetes and acidosis.

Materials and Methods. Animals. Male New Zealand white rabbits weighing from 1575 to 2400 g, housed in air-conditioned quarters, were fed standard chow and water *ad libitum.* Intravenous pentobarbital was used for anesthesia at the time of chamber placement and at sacrifice.

Alloxan diabetes. Alloxan diabetes was produced in the same manner as previously detailed (2) giving 200 mg/kg alloxan intravenously. The development of the metabolic abnormality was followed by repeated testing of the blood and urine for glucose and of serum and urine for ketones.[2] The results were recorded as: normal or elevated for blood glucose, negative or positive for glucosuria and negative, small, moderate or large for ketones.

Chamber. A 0.2 ml capacity plastic chamber was adhered with isobutyl 2-cyanoacrylate[3] over a wound produced by excision of a skin plug. Bleeding was controlled by cauterization. The chamber was filled with precision phosphate buffer (pH 7.381 ± 0.005 at 38°)[4] as used in Expt. B of the previous study (1).

pH determinations. The exudate and marginal ear vein blood from unanesthetized animals were collected in heparinized capillary tubes and the pH was determined with the microelectrode of a pH meter.[5] All blood and over half of the exudate pH determinations were done at least in duplicate. After determination of the pH of each blood or exudate sample the pH meter was tested with the standard precision buffers.

Histologic procedures. Fixation, staining and histologic study of the skin wounds excised at sacrifice were as previously described (3). A semiquantitative evaluation on a scale of 0 to 4+ of the overall intensity of inflammatory cell response, cellular exudation, and repair and regeneration was made for each lesion.

Experiment. Alloxan was administered after a base line blood pH and glucose determination and subsequently the animals were monitored for glucosuria and ketonuria. After appearance of glucosuria chambers were placed. Determinations of pH, glucose and ketones in exudate and blood were made at either 3, 6, 24, 48 or 72 hr from a total of 30 unanesthetized rabbits with ketosis of at least several hours duration. Subsequently the animals were sacrificed and the lesions were excised for histologic study. The 24 animals of Expt. B in the previous study (1), exam-

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

[2] Dextrostix, Clinistik and Acetest, Ames Company, Elkhart, IN.

[3] Supplied by Ethicon, Inc., Sommerville, NJ.

[4] Precision buffer type S1510, Radiometer, Copenhagen, The London Company, Cleveland, OH.

[5] Radiometer Astrup pH meter PHM27 and ultramicro pH electrode E5021, The London Company, Cleveland, OH.

ined over 96 hr and treated in a similar manner as those in the present experiment except for administration of alloxan, served as controls.

Results. Before alloxan administration the animals appeared healthy with normal blood glucose levels and a base line blood pH ranging from 7.287 to 7.450 with a mean of 7.375 (4). After alloxan the rabbits developed hyperglycemia and glucosuria within 24 to 48 hr followed shortly by ketonuria. Concomitantly the animals' health deteriorated so that long-term survival with untreated ketosis was not obtained. All animals studied at 3

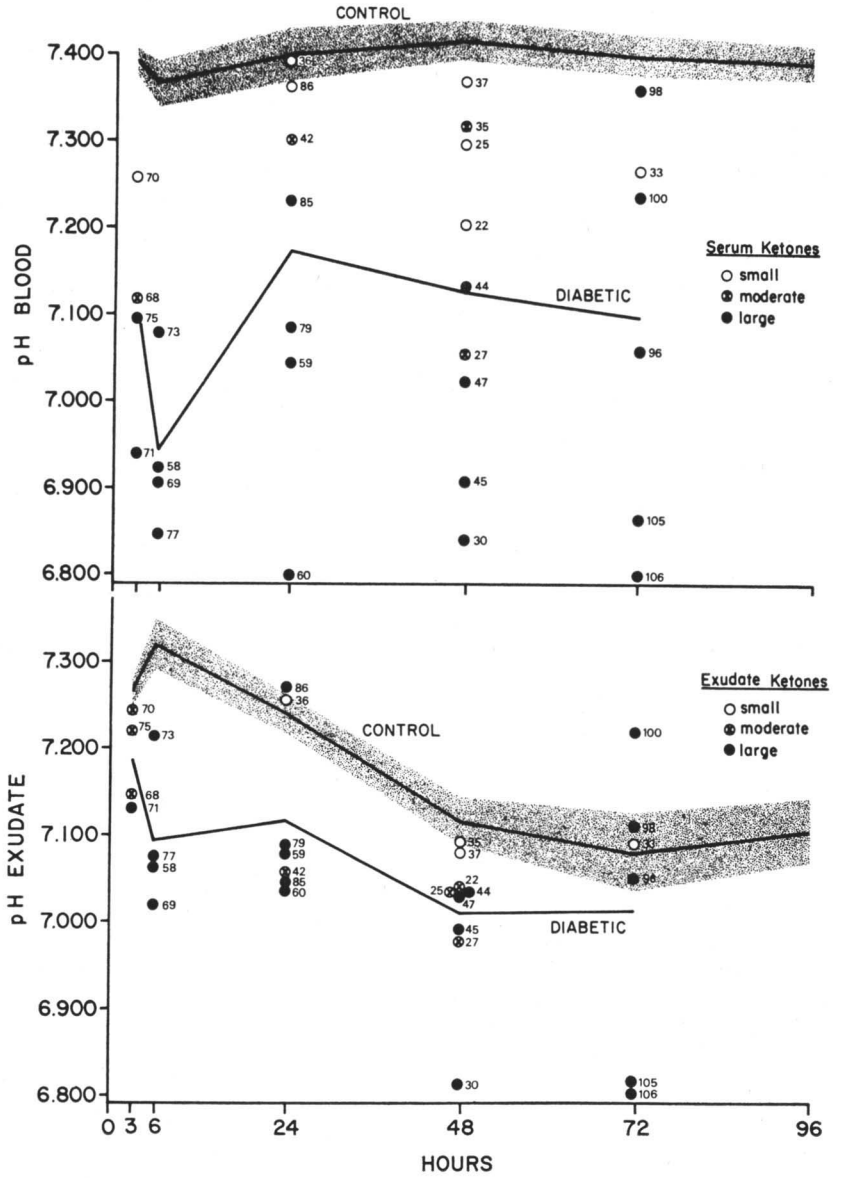

FIG. 1. Terminal blood and exudate pH values and ketone levels for each rabbit (identified by number). Mean (lines) and 95% CL (shaded area) of pH values from control and acidotic diabetic animals.

and 6 hr and 5 studied at 24 hr showed ketonuria both at the time of chamber placement and at exudate collection. Two animals at 24 hr and all studied at later times showed no ketonuria at the time of chamber placement but urinary ketones were demonstrated prior to exudate collection.

In the acidotic diabetic rabbits the terminal blood pH values showed a wide range. Most determinations revealed definite acidosis (Fig. 1) but in seven animals the blood pH values were within the base line range. The exudates (Fig. 1) also showed in most instances a local acidosis which was greater in the metabolically altered rabbits than that found in the controls. In general a correlation existed between the H-ion concentration in blood and exudate. The duration and severity of the ketonuria correlated well with the degree of acidosis in blood and exudate and the sickest appearing animals had the lowest pH values.

The inflammatory response in the wounds of the acidotic diabetic rabbits showed a marked and distinct decrease of the overall intensity of granulocytic margination, diapedesis and infiltration at 3 and 6 hr (Figs. 2 and 3). Reparative processes, namely fibroblastic and endothelial proliferation and epithelial regeneration were also affected but to a lesser degree and appeared delayed in time of onset and decreased in intensity. Many of the neutrophils in the lesions displayed even at the earliest times nuclear pyknosis and karyorrhexis. At 24 hr cellular margination and diapedesis were present to a degree similar to that of earlier times. At the later times exudation had subsided and the proliferative

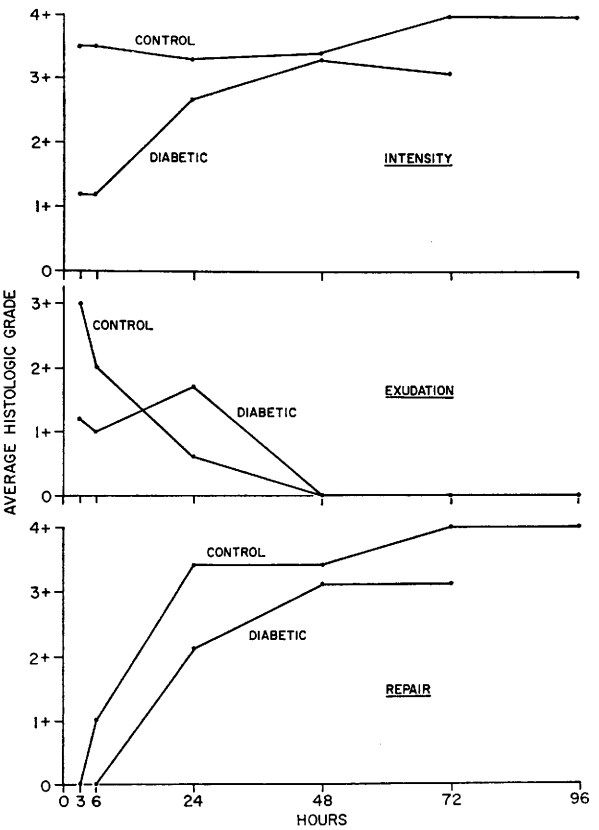

FIG. 2. Average histologic grade (scale of 0 to 4+) of overall intensity of inflammatory cell response, exudation (leukocytic margination and diapedesis), and repair and regeneration in skin lesions.

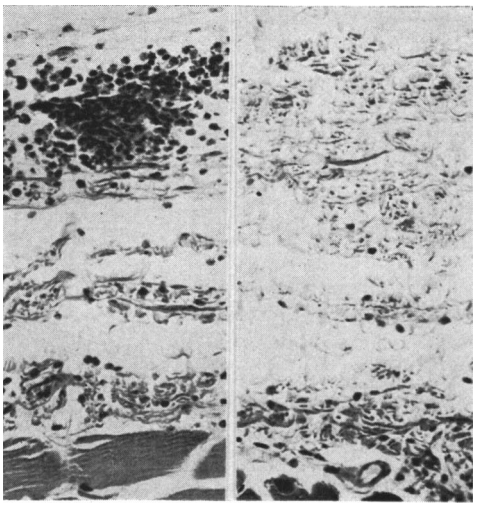

Fig. 3. Inflammatory cell infiltration at 6 hr: pronounced in control, left; virtually absent in acidotic diabetic rabbit, right. Giemsa, ×150.

and reparative processes were present.

In the metabolically normal controls (1) the pH of the exudate from the wound decreased with time and was lowest at 72 hr. The blood pH was unchanged during the course of the experiment. The inflammatory reaction in the wounds displayed the usual time of onset, intensity, duration and sequences of the exudative and proliferative cellular responses reflecting a sterile physical injury of brief duration.

Discussion. Multiple pH determinations from single samples of blood and exudate yielded closely corresponding values. The normal range of the blood pH was established by the base line determinations. In the acidotic diabetic rabbits the terminal blood pH values had a wide range and correlated well with the results of the terminal serum ketone determinations. Ketonuria served as a useful indicator to the level of the blood pH.

Significant acidosis of both blood and exudate was present in most of the alloxan-treated animals. At 6 hr blood acidosis was accentuated markedly in the acidotic diabetic animals and a similar but less marked lowering of the blood pH was also present at the same time in the controls (Fig. 1). This finding probably relates to the effects of anesthesia administered at the time of chamber placement. At the other times the blood H-ion concentration in the acidotic diabetic rabbits although significantly increased was less severely altered and the average values were similar to each other.

A local acidosis developed in the wounds of both acidotic diabetic and normal animals. With severe acidosis the blood and exudate pH values were generally similar. This direct relationship between blood and exudate pH was not usually seen in ainmals with mild degrees of acidosis where the inflammatory cell response and local acidosis resembled that seen in the normal animals. Determination of the average pH difference between blood and exudate showed a less marked diff-

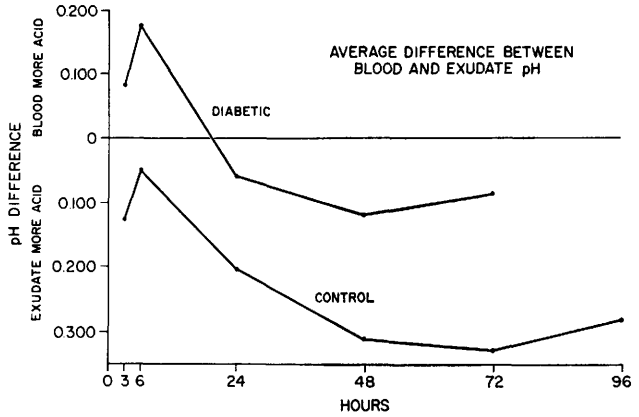

FIG. 4. Average differences between blood and exudate pH in control and acidotic diabetic animals.

erence in the acidotic diabetic rabbits than in the controls (Fig. 4). However, a parallel time course of changes was present in both groups.

The present findings confirmed earlier experimental and clinical observations (5–7) of an impairment of the acute inflammatory response in diabetic acidosis. The inflammatory cell response in the acidotic diabetic animals was delayed in onset and decreased in intensity when compared to that in normal controls. The less marked differences in inflammatory cell response between experimental and control animals at the later times probably reflected the absence of ketosis in the diabetic animals at the time of chamber placement.

The present data suggest that the exudate pH correlates with the intensity of the inflammatory cell response in the lesions. If the neutrophils of the inflammatory exudate are a source of the local increase of H-ion concentration then the decreased difference between blood and exudate pH in the acidotic diabetic animals might relate to the decreased inflammatory cell response of the diabetic animals.

Summary. The pH of inflammatory exudates collected at various times from chambers adhered over skin wounds of rabbits with acute alloxan diabetes and acidosis was determined. The H-ion concentrations of blood and exudate were compared with each other and with the inflammatory cell responses of the lesions. A local acidosis, indicated by low exudate pH, was present in all lesions. Significant local acidosis, similar to that observed in the lesions of normal rabbits, was present in the wounds of animals with lesser degrees of blood acidosis. Protracted severe blood acidosis was associated with similar low pH values in blood and exudates. The acute inflammatory cell response was delayed in onset and decreased in intensity in the severely acidotic diabetic animals. In these the presence of a markedly decreased acute inflammatory cell response and of a relatively less marked decrease of exudate pH suggest a possible relationship between the intensity of the acute inflammatory cell response and the H-ion concentration at the site of injury.

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

1. Edlow, D. W., and Sheldon, W. H., Proc. Soc. Exp. Biol. Med. **137**, 1328 (1971).

2. Bauer, H., Flanagan, J. F., and Sheldon, W. H., Yale J. Biol. Med. **28**, 29 (1955).

3. Hutchins, G. M., and Sheldon, W. H., Arch. Pathol. **90**, 377 (1970).

4. Altman, P. L., and Dittmer, D. S., (eds.), "Biology Data Book," p. 260. Prepared under the auspices of the Committee for Biological Handbooks, Fed. Amer. Soc. Exp. Biol., Washington, DC (1964).

5. Sheldon, W. H., and Bauer, H., J. Exp. Med. **110**, 845 (1959).

6. Sheldon, W. H., and Bauer, H., J. Exp. Med. **112**, 1069 (1960).

7. Perillie, P. E., Nolan, J. P., and Finch, S. C., J. Lab. Clin. Med. **59**, 1008 (1962).

Received Jan. 25, 1972. P.S.E.B.M., 1972, Vol. 140.