The pH of Inflammatory Exudates[1] (35782)

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The pH of an inflammatory reaction at the site of injury is thought to affect vascular permeability (1), the exudation, survival, and degradation of the white blood cells (2), phagocytosis (3), the state of the ground substance and mesenchyme (1, 4), the activity of complement (5), the function of enzymes and other chemical constituents (4), and the behavior of infectious agents (6, 7). Despite many investigations concerning most aspects of inflammation experimental studies of the H-ion concentration in inflammatory exudates are scant.

In previous clinical and experimental studies, chemical irritants or bacteria have been used to induce inflammation of tissues such as serous surfaces, skin, and lung where it is difficult to obtain a localized and standardized lesion of known duration (2, 8). The collection of samples for analysis and the techniques of pH determination have tended to introduce other sources of error (9). Despite these difficulties most investigators have found a distinct and sometimes marked increase of H-ion concentration in exudates and sites of inflammation (2, 8, 9). Decreased H-ion concentration has been reported in turpentine-induced pleural exudates of dogs (10). A detailed comparison of the results obtained by the various investigators is impractical because of wide variations in experimental design and methods.

The present study was designed to examine at various times and under uniform conditions the pH of inflammatory exudates and the histology of the inflammatory cell response. A sterile inflammatory reaction was elicited by a physical injury of short duration which produced a small open skin wound. A small chamber was adhered over the wound, the exudates were collected and the exudate pH was determined with a pH meter. The morphology of the inflammatory cell response in the lesion was studied histologically.

Materials and Methods. Animals. Male rabbits (New Zealand whites) weighing from 1300 to 2200 g were used. The animals, housed in air-conditioned quarters, were fed standard rabbit chow and water *ad libitum*. For placement of the chamber, the animals were anesthetized with intravenous pentobarbital, occasionally supplemented by inhalation of ether. Upon termination of the experiment the animals were sacrificed by air embolism while anesthetized.

Chamber. The chamber was similar to that already described (11) except for locating the outflow conduit on the top of the chamber (Fig. 1). A standard wound was produced by excision of a skin plug (11) and bleeding was controlled by cauterization.[2] The same number of cautery applications (8, each of 1-sec duration) were made to every wound even when bleeding did not occur. The chamber was adhered over the wound with isobutyl-2-cyanoacrylate.[3]

In the first experiment (A) the chamber was filled with an isotonic phosphate buffered saline solution (12) with pH 7.404 ± 0.015 at 38°. In the second experiment (B) a precision phosphate buffer[4] with pH 7.381 ± 0.005 at 38° was employed to fill the chamber.

Prior to collection of the exudate the exterior of the chamber was cleaned with 70%

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[2] Hildreth Coagulator E-915, Storz Instrument Company, St. Louis, Missouri.

[3] Supplied by Ethicon, Inc., Sommerville, New Jersey.

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

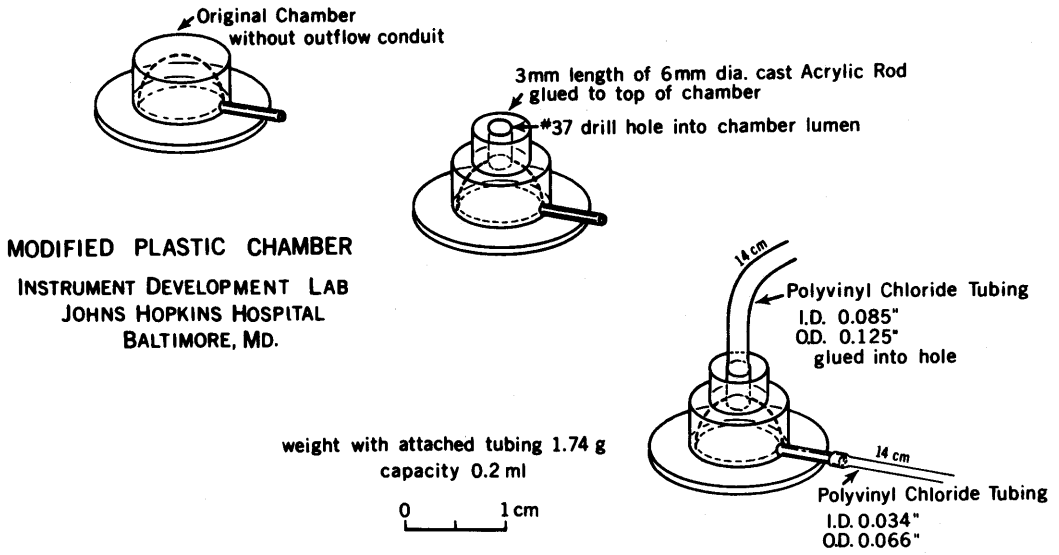

FIG. 1. Modifications of the original chamber.

alcohol and dried. The outflow conduit was cut off flush with the chamber top and the exudate was immediately aspirated into a heparinized capillary tube.

pH determinations. The exudate was drawn immediately from the capillary tube into the microelectrode of a pH meter[5] and the pH was read. In a similar manner, blood pH determinations were carried out on samples obtained from the marginal ear vein. Collection and reading of a sample were accomplished in less than 1 min. The pH determinations were done in duplicate and the collection of the exudate and blood samples was performed on unanesthetized animals.

Histologic procedures. Immediately after sacrifice, the skin wound and surrounding tissues were excised. Fixation, staining, and histologic studies were done as previously described (13). The histologic examination was a semiquantitative evaluation of time of onset, sequences, cellular composition, and intensity of the inflammatory cell responses including the morphologic aspects of repair and regeneration.

Experiment. Exudate and blood pH determinations were made at 3, 6, 24, 48, and 72 hr in Expt. A (15 rabbits, chamber filled with saline) and at identical times as well as at 96 hr in Expt. B (24 rabbits, chamber filled with precision buffer). Several animals were used for the study of each different time interval (Table I). Each animal was used only once, furnishing material from a single chamber for one set of exudate pH readings. Two sets of exudate pH determinations from two chambers placed at the same time on both sides of the back of a single animal were obtained once at 24 hr in Expt. A and once each at 6, 48, 72, and 96 hr in Expt. B. Terminal blood pH determinations were done in each animal within 5 min or less after the reading of the corresponding exudate pH. Initial base line blood pH values were obtained in all animals shortly prior to the placement of the chamber. After the exudate and terminal blood pH determinations, the rabbits were sacrificed; and the skin wounds were excised for histologic study.

Results. The animals remained healthy throughout the experiment. A majority of chambers (79%) yielded exudate sufficient for pH readings while in others (21%) fibrin clots and/or leakage prevented the collection of a sample.

The blood pH values prior to the placement of the chamber and at the time of the collection of the exudate in both experiments are shown in Table I. It appears that the

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

TABLE I. Number of Rabbits, Times of Sampling and Mean Values with 95% CL of Blood and Exudate pH in Expts. A and B.

Time (hr)	No. of rabbits	pH (mean and 95% CL)		
		Blood (initial)[a]	Blood (terminal)[b]	Exudate
		Expt. A		
3	3	7.417 ± 0.023	7.335 ± 0.065	7.286 ± 0.025
6	4	7.398 ± 0.024	7.401 ± 0.031	7.331 ± 0.021
24	2	7.397 ± 0.007	7.393 ± 0.006	7.247 ± 0.003
48	3	7.415 ± 0.011	7.403 ± 0.027	7.150 ± 0.080
72	3	7.393 ± 0.028	7.405 ± 0.008	7.007 ± 0.073
		Expt. B		
3	4	7.419 ± 0.020	7.392 ± 0.014	7.266 ± 0.015
6	4	7.416 ± 0.040	7.367 ± 0.027	7.320 ± 0.030
24	4	7.401 ± 0.034	7.403 ± 0.032	7.240 ± 0.022
48	4	7.432 ± 0.019	7.421 ± 0.024	7.116 ± 0.028
72	4	7.420 ± 0.020	7.404 ± 0.024	7.079 ± 0.046
96	4	7.396 ± 0.041	7.399 ± 0.019	7.106 ± 0.038

[a] Time of placement of chamber.
[b] Time of exudate pH reading.

experimental procedures did not affect the blood pH and that the method of pH determination was accurate and reproducible.

The exudate pH values and the corresponding blood pH readings at the various times are shown for both experiments in Table I and in Fig. 2. The blood pH is not altered. The exudate determinations at 3 and 6 hr show some drop of the pH. At 24 hr, the exudate pH is distinctly lowered and a fur-

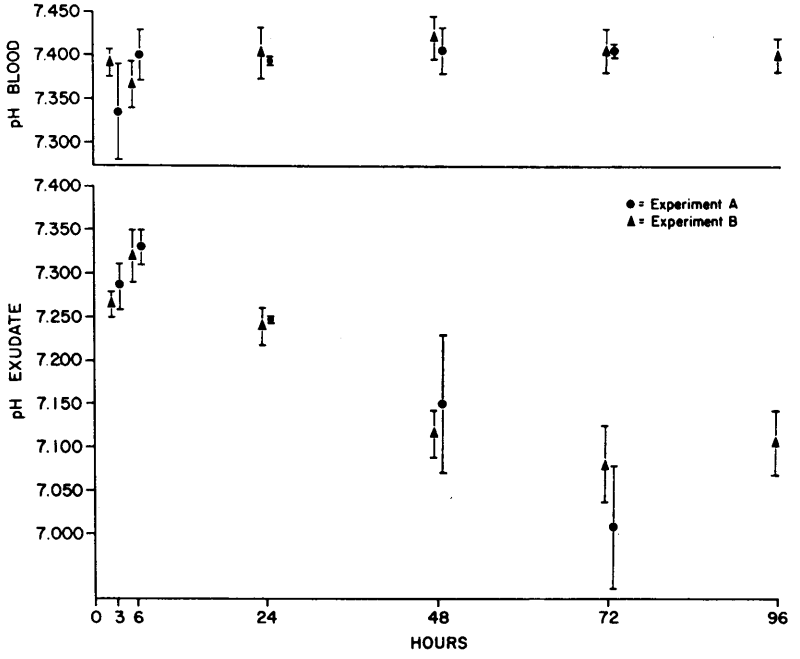

FIG. 2. Means and ranges of pH in Expts. A and B: (top) blood pH at time of exudate collection; (bottom) pH of exudates.

ther decrease is present at 48 hr. At 72 hr, the pH is lowest and there is no appreciable change at 96 hr.

In five rabbits, the exudate pH at five different time intervals was determined on the contents of two chambers carried by the same animal for the same period of time. The average difference between the pH values obtained from the two chambers was 0.049.

Histologic study showed no differences between Expts. A and B and revealed an inflammatory reaction of low intensity and short duration. A few small areas of coagulation necrosis related to the cauterization were present on the fundus and edges of the wound. Exudative processes with a predominance of neutrophilic granulocytes prevailed during the first 6 hr but had subsided by 24 hr. At this time and thereafter the principal morphologic findings consisted of reparative processes, namely proliferation of fibroblasts and endothelium, new formation of capillaries and epidermal regeneration. The cytoplasm of the regenerating cells was slightly basophilic. The fundus and edges of the wound showed at the later times a slight infiltration by mononuclear cells tending to collect around small veins. During the initial 24 hr, the exudate covering the wound fundus contained increasing numbers of neutrophils as well as some few fibrin strands. Degenerating neutrophils with cytoplasmic degranulation and cellular debris were numerous at 24 hr, less frequent at 48 hr, rare at 72 hr and not discernible thereafter. Vascular congestion was present during the first 6 hr and had disappeared later. Dilated lymphatics around the wound were present at 3 hr and thereafter.

Discussion. The animals tolerated the procedures well. The methods of producing a standard skin wound and of applying the chamber have become a routine procedure and in the majority of animals yielded sufficient material for analysis. The pH readings performed in duplicate and occasionally obtained from two chambers carried by the same rabbit have given closely corresponding values in both experiments and the results have been regarded as reliable.

There is evidence that the interior of the chamber adhered over an open wound communicates freely with the interstitial, vascular, and lymphatic compartments of the host. Significant quantities of various serum protein fractions as well as the cells of the inflammatory exudate rapidly accumulate in the chamber (11). Evans blue dye introduced into the chamber is shortly afterwards visible in the lymphatic vessels and the regional lymph node. Therefore analysis of the chamber contents appears to reflect the conditions in the underlying tissues and pH readings of the exudate are regarded as indicative of the H-ion concentration at the site of injury.

The solutions employed for filling the chambers were selected for the stability of the pH in order that the initial H-ion concentration with the chamber would be the same in each animal. The stability of the solutions had been ascertained by filling chambers adhered to siliconized microscopic slides and determining the pH at the time of filling and again 6 and 24 hr later. The results showed no change of pH.

The present findings demonstrate that the pH of the inflammatory exudate is lower than the blood pH which remains unaltered (overall mean pH 7.402). A definite decrease of the exudate pH is present at 24 hr (overall mean pH 7.242) and is most marked at 72 hr (overall mean pH 7.048) without appreciable variation at 96 hr. At 3 and 6 hr, the decrease in pH (overall mean value 7.274 and 7.328, respectively) is less distinct and these results may be a function of the buffering capacity of the solutions introduced into the chamber which somewhat mask an initial shift in H-ion concentration. The lowered pH of the inflammatory exudate in the absence of changes in the blood pH suggests an increased H-ion concentration localized at the site of injury.

In the present study, a sterile, local lesion was produced by a physical injury of short duration which evoked a slight inflammatory cell response. At 3 and 6 hr when the exudative cell response in the wounds was marked the exudate pH was only somewhat decreased although, as discussed above, the buffered solutions with which the chambers had been

filled may have modified early changes in the H-ion concentration. At 24 hr, when cell exudation had subsided, the exudate pH was distinctly decreased; and at 72 hr, when only reparative processes were seen in the lesions, the H-ion concentration of the exudate was greatest. A correlation between the cellular composition of the inflammatory response and the exudate pH was not demonstrated; and a relationship of the histologic changes, and particularly the exudative cell responses, in the lesions with the distinct changes of the exudate pH was not apparent.

The present findings correspond with those of most previous investigators who observed an increased H-ion concentration in inflammatory exudates (2, 8, 9). Generally the decrease of the pH described by others has been greater than that found in this study although a wide range of pH values from 8.3 (10) to 5.6 (14) has been reported. The differences may be related to the fact that in the present experiment exudates from small and rapidly subsiding inflammatory reactions of low intensity have been studied.

The decreased pH at the site of an inflammatory reaction has been attributed to the local increase in the concentration of lactic acid produced by the glycolytic activity of the polymorphonuclear leukocytes of the exudate (15). Our study neither indicates nor precludes a connection between either the granulocytes and other cells of the inflammatory cell response or other injured tissues at the site of inflammation. The present findings demonstrate an increase of the H-ion concentration, apparently confined to the wound which occurs early, is most marked after the exudative phase of inflammation has ceased and continues unchanged at a time when the lesion shows only reparative processes.

Summary. Inflammatory exudates were collected at various times in small chambers adhered over skin wounds of normal rabbits. The exudate pH was compared with the blood pH. At termination of each experiment the lesions were excised for histologic study. The blood pH remained unchanged. The pH of the exudate decreased with time showing a definite fall at 24 hr and reaching the lowest value at 72 hr without appreciable further change at 96 hr. The inflammatory cell response in the lesions was slight. Exudation of cells had subsided at 24 hr and thereafter only reparative processes were present. An increase of the H-ion concentration apparently confined to the lesion occurred within the first 24 hr of inflammation and persisted during wound healing. A correlation between the histology of the inflammatory reaction and the pH of the exudate was not apparent.

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