# STUDIES ON INFLAMMATION

## X. THE CYTOLOGICAL PICTURE OF AN INFLAMMATORY EXUDATE IN RELATION TO ITS HYDROGEN ION CONCENTRATION *

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For many years it has been known that the cytological sequence in acute inflammation is characterized in the earliest stages by an active emigration of polymorphonuclear leucocytes. After a time this is followed by an infiltration of mononuclear phagocytes. The latter have been designated by various names, the most satisfactory of which is perhaps that of "macrophage," originally suggested by Metchnikoff. In acute inflammation the polymorphonuclear cells that leave the circulating blood stream form the chief cellular constituents of the early exudate. The mononuclear phagocytes or macrophages increase in number in the later stages. These cells act as scavengers when the inflammatory irritant has been overcome. They engage actively in engulfing and digesting polymorphonuclear leucocytes, red cells, and various necrotic materials resulting from the acute inflammation. The orderly cytological sequence in the development of an inflammatory reaction was first pointed out by Borrel [1] and then by Durham [2] about forty years ago. The subsequent studies of Beattie [3] extended considerably the original observations of Durham. This sequence is true of the majority of inflammatory reactions caused either by bacteria or by chemical irritants. It is noteworthy that during the first twenty-four hours after their inoculation into normal tissues both tubercle and typhoid bacilli produce the same type of cellular changes as do various forms of pyogenic bacteria such as *Staphylococcus aureus*.[1, 4, 5] The difference in the leucocytic response found with various types of inflammatory irritants seems therefore to be one of degree rather than of kind.

No adequate explanation has been offered for this fundamental process. A number of years ago various investigators, particularly

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Opie, studied the action of intracellular proteolytic enzymes from leucocytes of an inflammatory exudate.[5, 6] Müller,[7] and subsequently Opie, showed that polymorphonuclear leucocytes contain an intracellular enzyme that acts in a slightly alkaline or neutral medium, but is almost wholly inactive in an acid reaction (0.2 per cent acetic acid). Opie designated this intracellular enzyme "leucoprotease." The action of this polymorphonuclear enzyme occurs only within the leucocyte, for in the plasma of an inflammatory exudate its activity is inhibited owing to the action of anti-enzymes. The earlier observations of Opie on the presence of antiferments inhibiting the action of leucoprotease have been recently confirmed by Weiss.[8] Opie furthermore demonstrated that the mononuclear phagocytes that accumulate in the later stages of the inflammatory reaction contain an enzyme causing active digestion of protein in a weakly acid medium, but almost entirely inactive at a neutral or alkaline reaction. The enzyme of the mononuclear phagocyte has been called "lymphoprotease."

It is conceivable that particles in an inflammatory exudate prior to being phagocytosed by a given type of leucocyte may tend to have on their surfaces a hydrogen ion concentration approximating that of the intracellular proteolytic enzyme capable of digesting them. If this assumption is correct then it is to be expected that the inflammatory exudate in which such particles are immersed would gradually increase in its acidity concomitantly with the shift from polymorphonuclear to mononuclear phagocytes. The question arises therefore as to whether or not there is a correlation between the pH of the medium and the cytological picture during the development of an acute inflammatory reaction. In this connection it is to be noted that Opie recorded several measurements on the reaction of a pleural exudate.[9] At no time during a 5 day period of the inflammatory process did the alkalinity of the exuded serum disappear; it was, however, less than that of the blood serum. It may be mentioned also that as the inflammation progressed there seemed to be a slight decrease in alkalinity. Since, however, no precautions apparently were taken to avoid loss of carbon dioxide during withdrawal and testing of the exudate, the validity of these measurements as absolute figures may be open to some question. Lord [10] in his studies on proteolytic enzymes in the pneumonic lung concluded that during the course of the disease a gradual increase

in the hydrogen ion concentration of the exudate probably occurs. He conceived resolution to be the result of this increased hydrogen ion concentration, which eventually activated a proteolytic enzyme having a range of optimum reactivity at a pH of 6.3 and 5.2.

Rous found that death of small cell aggregates resulted in the development of an alkalinity of these cells, owing to seepage into them of alkaline body fluids.[11] He recognized that the chemical changes that take place in small necroses differ in important respects from those occurring in large masses of dead tissue. He was led by his observations to conclude that very pronounced inflammatory edemas yield alkaline fluids but that "inflammation, as such, conduces to local acidosis."

None of the studies mentioned has correlated the pH of the inflammatory exudate with its differential leucocyte count. The object of the present communication is to report data on the trend of the hydrogen ion concentration and to relate this to the cellular changes in an exudate obtained at various intervals from an acute inflammatory area. The relation obtained suggests that the prevailing hydrogen ion concentration may be an important factor in determining at a given time the cytological picture of an inflammatory exudate.

## Experimental

*Method:* Pleural exudation was induced by the injection under ether anesthesia of 1.5 to 2 cc. of turpentine into the right chest of dogs.[9] Several hours to 1 day following the injection of the irritant a sample of the exudate was withdrawn by means of a Luer syringe with a hypodermic needle. The latter was of large caliber and filed off at the end, in order to diminish the chance of injury to the lungs. To prevent coagulation several glass beads were placed in the barrel of the syringe. When in one experiment about 0.5 cc. of 0.1 per cent heparin in Tyrode solution was employed as an anticoagulant, essentially the same readings were obtained as with the use of glass beads. Upon withdrawing the sample of exudate the syringe was shaken quickly for a few seconds and several smears were made on coverslips and slides. The remaining part of the exudate was immediately transferred under paraffin oil into a test tube.

Measurements of the pH were always performed within a short interval after withdrawing the sample of pleural exudate. The bicolor system of standards, as described by Hastings and Sendroy,[12] was employed in determining the hydrogen ion concentration. These investigators had found close agreement when results obtained by this method were checked up with parallel electrometric pH measurements. They also had determined that the "salt and protein errors" were negligible. The standards prepared with phenol red as indicator covered a range of pH 6.7 to pH 8. In a few instances the pH of the exudate was found slightly below 6.7. The reading in such cases was obtained

TABLE I

*The Hydrogen Ion Concentration and the Cytological Picture in Acute Inflammation*

| Dog No. | Interval between injection of irritant and removal of exudate | Differential leucocyte count of inflammatory exudate | | | pH of inflammatory exudate | Differential leucocyte count and pH of blood | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Polymorphonuclears | Lymphocytes | Mononuclear phagocytes | | pH | Polymorphonuclears | Lymphocytes | Mononuclears |
| | *hrs. : mins.* | *per cent* | *per cent* | *per cent* | | | *per cent* | *per cent* | *per cent* |
| 4 | 19:15 | 78.0 | 2.0 | 20.0 | 7.45 | | | | |
| | 43:45 | 78.0 | 1.0 | 21.0 | 7.23 | | | | |
| | 67:15 | 87.0 | 1.0 | 12.0 | 7.23 | | | | |
| | 93:08 | 31.0 | 3.0 | 66.0 | 6.97 | | | | |
| | 115:00 | 26.0 | 2.0 | 72.0 | 6.95 | 7.07 | 83.0 | 7.0 | 10.0 |
| 5 | 22:57 | 79.0 | 3.0 | 18.0 | 7.15 | | | | |
| | 47:00 | 9.5 | 0.5 | 90.0 | 6.8 | | | | |
| | 71:15 | 2.0 | 0.0 | 98.0 | 6.6 | 7.4 | 77.0 | 13.0 | 10.0 |
| 7 | 24:10 | 68.5 | 7.0 | 24.5 | 7.4 | | | | |
| | 48:30 | 72.6 | 3.4 | 24.0 | 7.35 | | | | |
| | 72:45 | 75.7 | 6.0 | 18.3 | 7.45 | | | | |
| 8 | 24:50 | 90.0 | 0.5 | 9.5 | 7.23 | | | | |
| | 47:35 | 87.0 | 2.7 | 10.3 | 7.13 | | | | |
| | 71:45 | 63.25 | 0.5 | 36.25 | 6.78 | | | | |
| | 100:05 | 59.0 | 1.0 | 40.0 | 6.98 | 7.1 | | | |
| 3 | 23:00 | 90.0 | 1.5 | 8.5 | 7.05 | | | | |
| | 48:10 | 53.5 | 1.0 | 45.5 | 6.75 | | | | |
| | 72:40 | 7.0 | 1.0 | 92.0 | 6.6 | | | | |
| | 95:40 | 10.0 | 3.0 | 87.0 | 6.65 | 7.23 | 71.0 | 7.5 | 21.5 |
| 2 | 23:42 | 69.3 | 8.3 | 22.3 | 7.4 | | | | |
| | 47:37 | 74.3 | 2.6 | 23.0 | 7.4 | | | | |
| | 71:12 | 83.0 | 2.0 | 16.6 | 7.25 | | | | |
| | 95:22 | 88.3 | 0.6 | 11.0 | 7.25 | | | | |
| 2-A* | 19:35 | .. | .. | .. | 7.23 | | | | |
| | 43:35 | 13.0 | 3.0 | 84.0 | 6.6 | | | | |
| | 67:10 | 3.5 | 0.5 | 96.0 | 6.7 | 7.23 | 77.0 | 10.0 | 13.0 |
| 10 | 6:15† | .. | .. | .. | 7.55 | | | | |
| | 24:25 | 40.5 | 12.5 | 47.0 | 6.93 | | | | |
| | 48:15 | 63.5 | 11.5 | 25.0 | 7.0 | | | | |
| | 72:38 | 74.0 | 6.0 | 20.0 | 7.08 | | | | |
| | 96:15 | 74.0 | 2.0 | 24.0 | 7.28 | | | | |
| | 120:15 | 76.0 | 0.0 | 24.0 | 7.28 | | | | |
| | 145:35 | 74.0 | 3.0 | 23.0 | 7.50 | | | | |
| | 169:35 | 78.5 | 7.5 | 14.0 | 7.55 | | | | |
| 11 | 24:15 | 26.0 | 9.75 | 64.25 | 6.98 | 7.28 | 69.0 | 18.0 | 13.0 |
| 9 | 52:55 | 72.0 | 6.0 | 22.0 | 7.05 | 7.05 | | | |
| 16 | 18:00 | 71.0 | 10.0 | 19.0 | 7.55 | 7.28 | 83.0 | 4.0 | 13.0 |
| 12‡ | 23:30 | 81.0 | 1.5 | 17.5 | 7.25 | | | | |
| | 48:35 | 52.0 | 1.0 | 47.0 | 6.95 | | | | |
| 14‡ | 23:25 | 90.0 | 0.66 | 9.33 | 7.2 | | | | |
| | 47:20 | 64.0 | 4.5 | 31.5 | 6.95 | | | | |
| | 75:20§ | 87.0 | 0.0 | 13.0 | 7.28 | | | | |
| 13¶ | 24:06 | 87.5 | 2.0 | 10.5 | 7.4 | | | | |
| | 49:02 | 77.5 | 2.5 | 20.0 | 7.5 | | | | |
| | 71:45 | 74.0 | 6.0 | 20.0 | 6.98 | | | | |
| | 95:30 | 10.0 | 8.5 | 81.5 | 6.8 | 7.45 | 76.0 | 9.0 | 15.0 |
| 15¶ | 23:25 | 86.0 | 1.0 | 13.0 | 7.35 | | | | |
| | 47:15 | 42.5 | 2.0 | 55.5 | 6.8 | | | | |
| | 75:40 | 17.5 | 3.5 | 80.0 | 6.75 | | | | |

* Dog 2-A is Dog 2 reinjected with turpentine in the right pleural cavity 35 days after the first injection with the irritant.

† Upon removal of the exudate 6 hours and 15 minutes after the injection of the irritant practically no leucocytes were found.

‡ A total of 8 to 9 cc. of a phosphate buffer mixture at pH 6.78 was injected in divided doses, subsequent to the irritant, into the right pleural cavity.

§ This particular sample of the exudate was removed immediately after killing the animal.

¶ A total of 15 to 20 cc. of a phosphate buffer mixture at pH 7.28 was injected in divided doses, subsequent to the irritant, into the right pleural cavity.

by the use of bromcresol purple as indicator and represents only a first approximation. The thorough studies of Drury and Rous had shown that in the animal body, at least, the observed colors in tissues vitally stained with phenol red or bromcresol purple cannot be ascribed to indicator errors resulting from association of the phthalein with tissue materials.[13] For this reason it is believed that the readings obtained by adding under oil 0.2 cc. of the pleural exudate to 4 cc. of a standard phenol red indicator solution (made up in saline and adjusted to about pH 7.4) are reasonably reliable and do not represent indicator errors. In several instances, in spite of an appropriate saline control tube, the turbidity of the exudate rendered readings somewhat difficult, so that centrifugalization for a few minutes had to be resorted to. The determinations were always made after the tubes had been immersed in a water bath at about 38° C for several minutes.

The differential leucocyte counts were made from smears on coverslips and slides. The cells were stained by the Wright method. As a rule several hundred cells were counted in each sample. In computing the percentage of polymorphonuclears and mononuclears cells were frequently encountered that were so degenerated as to render their identification difficult. These were not included in the final counts.

Samples of the pleural exudate were withdrawn daily and studied as described for a period not exceeding 1 week after the injection of the irritant. At the completion of an experiment the animal was anesthetized under ether and frequently a sample of blood was withdrawn from the femoral vein in a syringe containing about 0.5 to 1 cc. of 0.1 per cent heparin in Tyrode solution. The pH of the blood and its differential leucocyte count were determined. The administration of ether was continued until the death of the animal. A postmortem examination was performed and specimens of the inflamed pleura and right lung were placed in 10 per cent formaldehyde for subsequent histological examination.

## Results

The results of all the 15 experiments performed are summarized in Table I. A cursory examination of the data shows that in 8 out of 12 animals in which the pleural exudate was studied from day to day, as the inflammatory reaction progressed, the hydrogen ion concentration changed from an alkaline to an acid pH. The change in the reaction toward a definite acidity occurs usually 2 or 3 days after the injection of the irritant. Concomitantly with this decrease in the alkalinity of the exudate there is a change in the differential leucocyte formula. The percentage of polymorphonuclears falls, whereas the percentage of mononuclear phagocytes correspondingly rises. The percentage of lymphocytes evidently plays no significant rôle in these cellular changes. Composite graphs of all experiments showing the parallelism in the fall of the pH and the drop in the percentage of polymorphonuclear leucocytes appear in Chart 1.

Since the percentage of polymorphonuclear leucocytes represents virtually the reciprocal of that of the mononuclears, the latter were not plotted on the chart. An examination of the data reveals that the percentage of polymorphonuclears predominates over that of the mononuclears whenever the pH is alkaline. A rise in the hy-

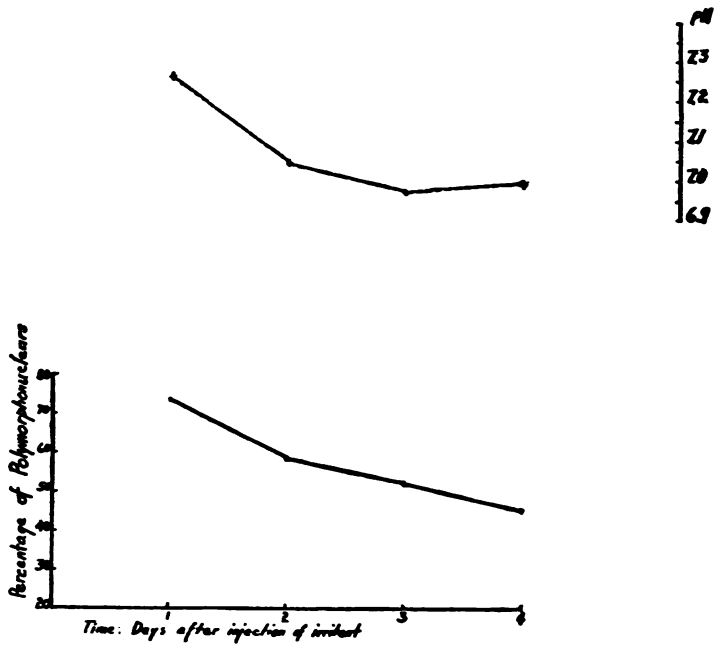


CHART 1

The hydrogen ion concentration in relation to the percentage of polymorphonuclear leucocytes in pleural inflammatory exudates. Composite graphs of 12 experiments.

– – – – – + – – – – – pH
———— • ———— Percentage of polymorphonuclear leucocytes.

drogen ion concentration is immediately or at least very soon followed by a fall in the percentage of polymorphonuclear leucocytes. By studying the hydrogen ion concentration one can fairly well predict the cytological picture in the exudate, and *vice versa*. The correlation is evidently very close. In Dogs 7, 2, and to some extent in Dog 10 the pH failed to become acid concomitantly with the progress of the inflammatory reaction. The counts correspondingly reveal a predominance in the percentage of polymorphonuclear cells throughout the period of the experiments (Chart 2, Dog 7).

In Dog 11 the per cent of polymorphonuclears appears surprisingly low for an inflammation of only 24 hours duration; the pH here is 6.98. To summarize, the point under discussion can perhaps be illustrated by the following calculation from Table I. In 31 counts in which the percentage of polymorphonuclears ranged from 60

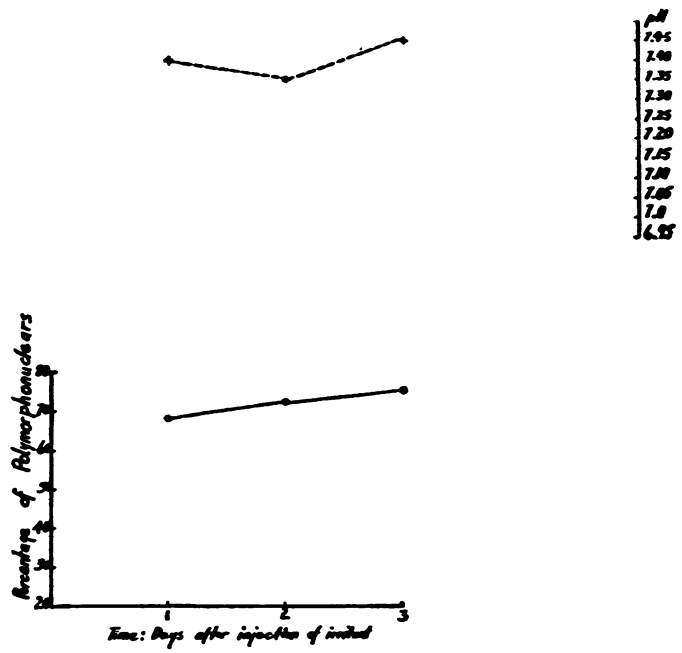


CHART 2

The hydrogen ion concentration in relation to the percentage of polymorphonuclear leucocytes in pleural exudation from Dog 7. Note that the pH remains alkaline and that the percentage of polymorphonuclears maintains a high level throughout the duration of the experiment.

– – – – – + – – – – – pH
——————·—————— Percentage of polymorphonuclear leucocytes

to 90 per cent, the pH averaged 7.25. Contrast this with 16 counts in which the percentage of polymorphonuclears ranged from 2 to 60 per cent, and the pH averaged 6.80.

Having obtained definite evidence of the close parallelism between changes in hydrogen ion concentration and in the differential leucocyte formula in acute inflammation the question arose as to which comes first, the changes in the pH or the cellular modifica-

tions. The present data are highly suggestive in answering this question. An examination of the results obtained in the case of some individual experiments points out that the increase in the hydrogen ion concentration evidently precedes the fall in the percentage of polymorphonuclear leucocytes. Chart 3 illustrates this

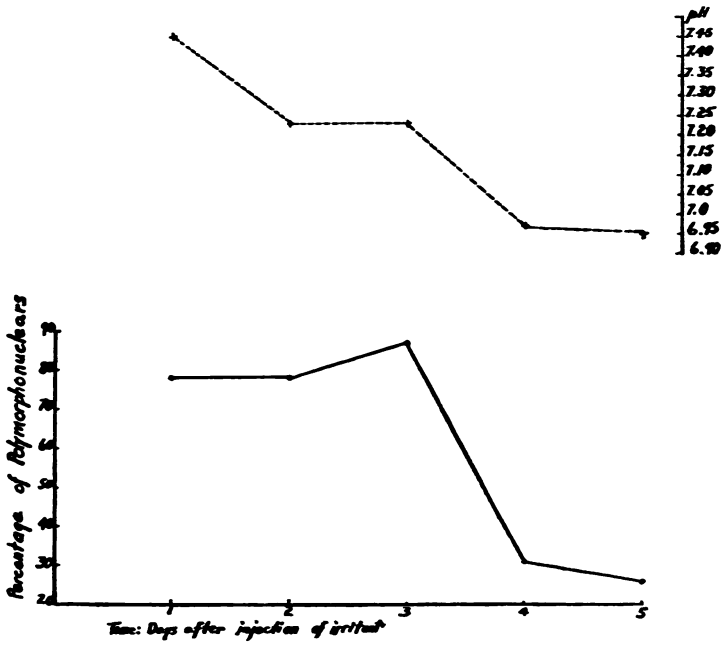


CHART 3

**The hydrogen ion concentration in relation to the percentage of polymorphonuclear leucocytes in pleural exudation from Dog 4. Note that the pH steadily declines during the first 2 days, while the percentage of polymorphonuclears remains at a high level.**

– – – – – + – – – – – pH
——————•—————— Percentage of polymorphonuclear leucocytes

fact to some extent in the case of Dog 4. Whereas the pH of the exudate steadily declines from an initial value of 7.45, the percentage of polymorphonuclear leucocytes remains high. There was an abrupt fall in the percentage of polymorphonuclears only when the pH reached 6.97. The point is perhaps better exemplified in the case of Dog 13, Chart 4. For the first 2 days the pH was alkaline, 7.4 and 7.5 respectively. The percentage of polymorphonuclears was high, 87.5 and 77.5. On the 3rd day there was an abrupt fall

in pH to 6.98. The percentage of polymorphonuclears, however, was still high, namely 74. On the 4th day the pH was lower than on the preceding day, namely, 6.8. The exudate contained only 10 per cent of polymorphonuclears. Hence in this experiment the sharp rise in hydrogen ion concentration definitely preceded the fall

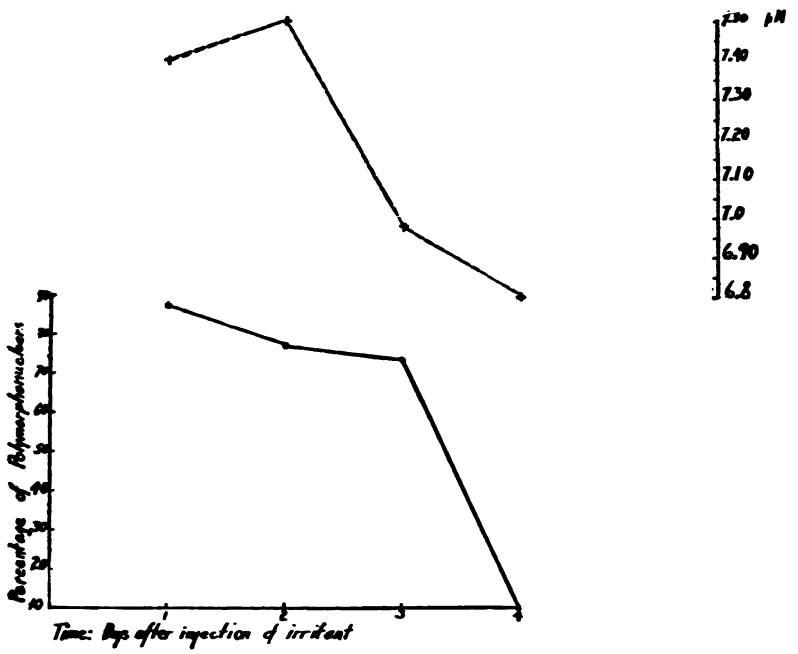


CHART 4

The hydrogen ion concentration in relation to the percentage of polymorphonuclear leucocytes in pleural exudation from Dog 13. Note that the abrupt fall in the pH precedes the sharp drop in the percentage of polymorphonuclear leucocytes.

- - - - - + - - - - - pH
——————·—————— Percentage of polymorphonuclear leucocytes

in the percentage of polymorphonuclear leucocytes. The latter followed the decrease in alkalinity only after the lapse of a definite period. This is definite evidence that the fall in pH precedes the changes in the cytological picture. The hydrogen ion concentration may thus possibly be the regulating factor in determining the differential leucocyte formula of an exudate. In view of what is known of the mechanism of intracellular enzyme action in leucocytes, a physicochemical regulatory mechanism of this type would not be

wholly unexpected. That the fall in pH seems to precede the drop in the percentage of polymorphonuclear leucocytes is quite evident from the above analysis. At the same time it is obvious on examining the data that this relation is not always evident. This seems to depend on the rapidity of the change in reaction. If the rise in hydrogen ion concentration is rapid and sharp the corresponding fall in the percentage of polymorphonuclears may occur so rapidly as to appear to be a parallel phenomenon (see Dog 15, Table I, Chart 5). When the change in reaction proceeds very rapidly the exudate smears invariably reveal numerous degenerated, swollen, and vacuolated polymorphonuclear leucocytes containing characteristically fragmented and intensely stained nuclei. Such lethal effects accompanying an abrupt change in the reaction with increase in the acidity may be an important factor in explaining suppuration at the site of inflammation. In this connection it is perhaps also interesting to note that Rous [11] in his studies on factors that determine the reaction of skin grafts came to the conclusion that the developing acidity, in the initial stages at least, when the graft was isolated from its surroundings, was referable to the elements of the tissue proper. (Almost no cells had wandered into the grafts at this time.)

Further experiments were undertaken in an endeavor to modify experimentally the pH of an inflammatory exudate and to determine the effect of such procedure on the differential leucocyte formula. Phosphate buffers (Sörensen) were prepared at pH 6.78 and 7.28. Several cubic centimeters of each of these buffer solutions were injected immediately after the introduction of turpentine into the right chest of dogs. The phosphate buffers were reinjected at intervals of several hours. The periodic withdrawal of pleural exudates showed, however, the same tendency toward an ultimate acidosis and rise in the percentage of mononuclear phagocytes (see Dogs 12, 13, 14, 15, Table I). It became clear that the buffering mechanism of the tissues at the site of inflammation was not easily influenced by the mere introduction of phosphate buffer solutions.

Postmortem examination of the right chest of dogs injected with turpentine several days previously revealed an intense serofibrinous and at times a fibrinopurulent exudate. The pleura was greatly thickened and fibrinous adhesions extended from the visceral to the parietal layers, thus forming small pouches in the pleural cavity. These contained various amounts of exudate. In agreement with

histological studies by Opie [9] such tissues provided in general the same type of information regarding the cellular infiltration as was obtained from stained exudate smears.

It was of some interest to note that animals which throughout the experiment maintained an alkaline exudate (Dogs 7, 2, and 10)

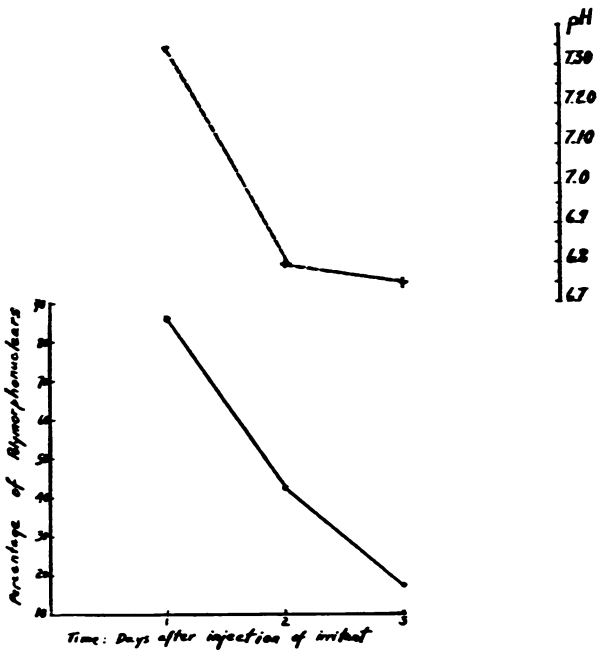


CHART 5

The hydrogen ion concentration in relation to the percentage of polymorphonuclear leucocytes in pleural exudation from Dog 15. Note the abrupt fall in the pH and in the percentage of polymorphonuclear leucocytes on the 2nd day following the injection of the irritant.

– – – – – + – – – – – pH
——————·—————— Percentage of polymorphonuclear leucocytes

appeared in much better physical condition than those whose pleural exudate gradually became acid in reaction. In the latter, dyspnea, weakness and general apathetic behavior were not infrequent.

The effect of reinjection of the same irritant was tried in the case of Dog 2. This animal received 2 cc. of turpentine intrapleurally. Its pleural exudate remained alkaline and showed a high percentage of polymorphonuclear leucocytes for 4 days. The animal was in

perfect condition at the end of the experiment. Thirty-five days later he was reinjected intrapleurally with 2 cc. of the same irritant. Within 2 days the pH was 6.6 and there was an overwhelming number of mononuclear phagocytes in the exudate (Dog 2-A, Table I). The acid pH persisted and on the 3rd day when the experiment was terminated the animal displayed some difficulty in breathing. The pH of the blood was 7.23 and contained 77 per cent of polymorphonuclear leucocytes. In a previous communication the writer [14] pointed out that an area of inflammation is ultimately walled off from the rest of the organism; the inflamed area develops its own local circulation, its own hydrogen ion concentration and its own metabolism. This view is substantiated when the data on the pH of the blood are compared with those obtained in the majority of exudates in the later stages of the inflammatory reaction (Table I).

## Discussion

The results obtained in this series of experiments reveal the fact that in most instances an acute pleural inflammation induced by a strong chemical irritant such as turpentine gradually develops a local acidosis. Furthermore, the data point toward a definite relation between the hydrogen ion concentration and the cytological picture of an inflammatory exudate. The findings certainly indicate the occurrence of a parallel between the hydrogen ion concentration of the fluid medium and that favorable to the action of the enzyme of the predominating phagocyte. The relation between the two phenomena is strongly suggested by the following considerations.

In the first place, although inflammation as such conduces to local acidosis with a concomitant shift in the cell counts from polymorphonuclear to mononuclear cells, it is interesting to note that in several experiments an alkalinity was maintained throughout the period of the inflammation (see Dogs 7, 2, 10, Table I). In such cases, and only under these conditions, was there no shift in the cell counts, the percentage of polymorphonuclears remaining at a constantly high level even in the later stages of the inflammatory process. It becomes somewhat difficult to consider this state of affairs mere coincidence. Secondly, the fact that by determining the pH of the exudate the character of the cytological picture could be

fairly well predicted and *vice versa* seems to be definite evidence of some correlation between the pH and cell count. In the third place, the fact that in a few instances when the shift from alkaline to acid took place rapidly the cell change, although delayed, nevertheless invariably followed appears to warrant the inference that if there is an interdependence it is the pH that conditions the cytological picture and not the reverse order of sequence. The observations reported in this communication seem therefore to support the conclusion that the differential leucocyte picture at a given time in the development of an inflammatory reaction is a function of the pH of the exudate.

The implications of this concept are obvious. It is possible that an understanding of the histological differences of various inflammatory lesions may be facilitated through a study of their respective hydrogen ion concentrations.

Opie [5] pointed out that the studies on intracellular enzymes of leucocytes have served to explain many of the phenomena of resolution. Some of his earlier conclusions [15] on the solution of tissue with abscess deserve perhaps revision, in view of the present observations. Briefly stated, Opie's original experiments on abscess formation consisted in inducing a purulent exudation by the subcutaneous injection of turpentine. Four or 5 days later a large cavity distended with fairly thick purulent fluid was formed. The cells of this pus were separated from the serum by centrifugalization. To the cell-free pus serum, leucoprotease was added. This combination freely digested coagulated serum. On the other hand, the same polymorphonuclear enzyme in the presence of blood serum failed to digest materially the coagulated serum. From these facts Opie concluded that the anti-enzymatic action of a limited quantity of exuded serum is overcome by an increasing quantity of proteolytic enzyme set free by disintegration of polymorphonuclear leucocytes, thus accounting for the solvent effect on tissues of a purulent exudation. This conclusion is now perhaps somewhat difficult to accept; at least the data presented in this paper open the way to a different interpretation. It has been found (Table I) that as a rule 4 or 5 days following the onset of an inflammatory process induced by turpentine the resulting purulent exudate is usually characterized by an acid reaction. Opie has demonstrated that both leucoprotease and anti-enzymes are inactive in an acid medium. It is therefore

more likely that the solution of fibrin or necrotic tissue in a purulent area of inflammation is due to the activation of autolytic enzymes by the acid reaction. Enzymes of this type have been adequately described in a recent review by Bradley.[16] This interpretation appears to be more in accord with the facts since it is hardly possible to assume in view of Opie's own findings that an excess of leucoprotease from disintegrating polymorphonuclears could act in an acid medium. It seems probable that in Opie's experiment the proteolytic enzyme, leucoprotease, was inactivated by the acid cell-free pus, while at the same time there were present in this fluid autolytic tissue enzymes that possessed an optimum activity in an acid pH and were hence able to digest the coagulated serum. Opie's earlier view on the mechanism of caseation, which he considered to be the result of an accumulation of autolytic enzymes released from epithelioid cells and which acted in an approximately neutral or weakly acid medium, is not wholly dissimilar to the writer's view, as just expressed in regard to the solution of fibrin and necrotic tissues in an abscess.[17] Furthermore, it is to be noted that Opie himself pointed out in some of his later studies [9] that whereas leucoprotease may play a part early in the digestion of fibrin, the latter undergoes solution in the advanced stages of an inflammatory reaction only in the presence of weak acid. Opie [5, 9] therefore concluded that fibrin is ultimately digested by an enzyme having the character of lymphoprotease and resembling the autolytic enzymes of tissues. In an endeavor to throw further light on the question under discussion 1.5 cc. of turpentine were injected subcutaneously into the right flank of a dog. Four days later a large subcutaneous abscess containing thick viscous pus resulted. The pH of this exudative material was definitely acid in reaction, approximately 6.6. This would support the contention that an excess of leucoprotease could not possibly be the important factor in the solution of tissues in such abscesses since this enzyme is active only in an alkaline or neutral medium.

Bayliss,[18] as a result of his studies on emulsin, expressed considerable doubt as to the actual existence of true anti-enzymes as follows: "Some of the effects described as being due to them are to be accounted for by changes of hydrogen ion concentration, others to adsorption of the enzyme by a colloid." Bayliss pointed out that in his emulsin experiments the effect was found to be due merely to

diminution of the acidity of the solution. The inhibitory effect of the anti-enzyme disappeared when the solution was brought back to the initial value by the addition of acid phosphate. This idea may doubtless have considerable importance in revising our accepted concepts concerning so-called "anti-enzymes" in inflammation, especially in view of the progressive increase in hydrogen ion concentration in such pathological areas. Nevertheless, although Bayliss may be correct as far as anti-enzymes are not comparable in regard to specificity to the true antibody, still by counteracting the effectiveness of the enzyme, whether by adsorption or by changes in the hydrogen ion concentration, the anti-enzymatic effect of serum on enzymes remains a fact.

The mechanism conducing to local acidosis in inflammation is still somewhat problematical. Schade and his co-workers [19] reported that pus from acute abscesses had a pH ranging from 5.95 to 6.50; the pH of normal tissue fluids ranging from about pH 7.10 to pH 7.40. The studies of Irisawa [20] and of Ito [21] have shown that lactic acid is a constant constituent of pus. Gessler [22] has demonstrated that the oxygen consumption and the metabolic rate are increased in an inflamed area. This state of affairs would doubtless favor the development of a local acidosis unless properly compensated by an equally increased and effective fluid circulation at the site of inflammation. The writer has shown in previous studies that various foreign substances, including bacteria and electrolytes, are unable to escape readily from the site of inflammation owing to the presence of a fibrinous network and of thrombosed lymphatics. Furthermore, in acutely inflamed areas of moderately long standing a number of vascular capillaries have also been found with their lumina occluded by thrombi.[32] It is conceivable, therefore, that as acid metabolites are formed in an acutely inflamed area these tend to be fixed *in situ*, thus causing a rise in the hydrogen ion concentration of the exudate.

## Summary and Conclusions

A pleural inflammatory exudate, in the majority of instances, develops a rise in its hydrogen ion concentration concomitantly with the progress of the inflammatory reaction.

When the pH of the exudate is alkaline the percentage of poly-

morphonuclears at the site of inflammation exceeds that of the mononuclear phagocytic cells.

When the pH of the exudate is approximately neutral the percentage of polymorphonuclear cells tends to approach that of the mononuclear phagocytes.

When the pH of the exudate is definitely acid large numbers of polymorphonuclear cells are found degenerated. The percentage of relatively normal appearing polymorphonuclear leucocytes is found considerably lower than that of the mononuclear phagocytes.

In some cases the pH of the exudate remains alkaline throughout the period of an acute pleural inflammation. In these instances the percentage of polymorphonuclears invariably exceeds that of the mononuclears.

By measuring the hydrogen ion concentration of an inflammatory exudate the character of the cytological picture can be predicted with a fair degree of certainty. Likewise the converse follows.

Evidence has been obtained to show that the development of a local acidosis in an area of inflammation precedes at times the changes occurring in the differential leucocyte formula of the exudate. In such cases, however, the cytological changes ultimately follow the development of the acid reaction.

The observations reported suggest that the differential leucocyte formula in an area of acute inflammation is a function of the hydrogen ion concentration of the exudate. The cytological picture in an inflamed area seems to be conditioned by the pH of the exudate surrounding the injured tissue. The present study indicates that the developing local acidosis as the inflammatory reaction progresses can adequately account for the shift in infiltration from polymorphonuclear leucocytes to mononuclear phagocytes at the site of inflammation.

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