# STUDIES ON INFLAMMATION *

## XIII. Carbohydrate Metabolism, Local Acidosis, and the Cytological Picture in Inflammation

Valy Menkin, M.D., and Charlotte R. Warner

*(From the Department of Pathology, Harvard University Medical School, Boston, Mass.)*

Earlier studies by one of us [1] have indicated that the cellular picture in an area of inflammation is apparently a function of the local hydrogen ion concentration. The usual cytological sequence in the development of the acute inflammatory reaction consists of an initial infiltration of polymorphonuclear cells that are subsequently replaced by an abundance of macrophages. This change in the cellular elements is correlated by the development of a local acidosis at the site of inflammation. In the initial phase, when the polymorphonuclear cells predominate, the pH of the exudate is definitely within an alkaline range (about 7.3 to 7.4). With the progress of the inflammatory reaction the pH may drop to 6.7 or approximately 6.5. Polymorphonuclear cells for the most part seem unable to survive, without being injured, a pH below 7.0. The predominance of mononuclear phagocytes is conspicuous when the pH of the exudate falls to a level ranging between 7.0 and about 6.8. At greater hydrogen ion concentration, as found in frank pus, all or most types of leukocytes appear to be injured. At times the pH of the exudate remains alkaline throughout the period of acute inflammation. This is invariably attended by a maintenance of the polymorphonuclear phase, indicating thus a probable interrelation between the hydrogen ion concentration and the cellular elements of an exudate. In brief, by determining 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 obtained in a previous study showed that the development of local acidosis in an area of inflammation precedes at times the changes occurring in the differential leukocyte formula of the exu-

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date.[1] In such cases, however, the cytological shift to the macrophage stage ultimately follows the development of the acid reaction.

The purpose of this communication is to present observations on the mechanism of local acidosis in inflammation. The elucidation of this mechanism would probably be of distinct aid to a basic understanding of some of the factors controlling the histological manifestations of a variety of infectious lesions. The observations presently to be described indicate the important rôle of the local carbohydrate metabolism in determining the hydrogen ion concentration of an inflamed area.

Since the classical work of Fletcher and Hopkins [2] on the production of lactic acid in amphibian muscle, numerous publications and reviews have appeared dealing with the chemistry of muscular contraction.[3,4] The Meyerhoff-Hill theory on the rôle of lactic acid in the contractile phase has been recently subjected to severe revision owing to the contributions of Embden and Lawaczeck,[5] Fiske and Subbarow,[6,7] and Eggleton and Eggleton,[8] which led to the discovery of phosphocreatine. These investigators pointed out the important relation of phosphate compounds to the chemical dynamics of muscular contraction. It is well known that other tissues besides muscle manifest glycolytic reactions. Gerard and Meyerhof [9] demonstrated that the frog nerve under anaerobiosis forms lactic acid which on readmission of oxygen disappears.[10] Brain tissue, containing a negligible amount of glycogen, forms lactic acid directly from blood sugar.[11] When maintained outside of the body in a milieu deprived of oxygen most tissues form lactic acid from glucose, *i.e.* through the process of glycolysis. Malignant tissue, normal retina and embryonic tissues display a strikingly high rate of glycolysis. Warburg, Posener and Negelein [12,13] pointed out that of a variety of tissues kept in blood plasma and fully oxygenated, only tumor tissue, and particularly malignant tissue, was capable of producing large amounts of lactic acid. This distinction is not strictly correct. There are normal tissues, such as mammalian erythrocytes, which glycolyze in spite of an adequate oxygen supply. Furthermore, Crabtree [14] demonstrated that hyperplastic lymph nodes and inflammatory granulations caused either by tubercle bacilli or by filterable viruses likewise show pronounced aerobic glycolysis. Scheller [15] found that exudates from suppurative lesions display considerable glycolytic activity when incubated for several hours.

Jervell [16] made a series of extensive investigations on the concentration of lactic acid in blood and urine under various pathological states. He pointed out that the normal "oxygen debt" following muscular activity is always present, even during repose, in patients with decompensated cardiac conditions. This explains their unfitness for physical exertion. In anemia, in pneumonia with partial asphyxia, or in advanced pulmonary tuberculosis, hyperlactacidemia is frequently encountered. From these observations Jervell concluded that an augmentation in lactic acid in the venous blood indicates a deficient oxygen supply to the tissues. The consequent incomplete oxidations lead to an accumulation of lactic acid.

One of us has pointed out in earlier studies that the development of an acute inflammatory lesion involves a walling-off process owing to blockage of normal lymphatic drainage.[17] With the initial increase in capillary permeability and the passage of plasma proteins into tissue spaces, the local circulation becomes seriously impaired as evidenced by the "packing" of cells in the capillary lumen. Thrombosis may induce partial or complete obliteration of the vascular channels at the site of injury. The formation of a fibrinous network in the extracapillary spaces restricts the free diffusion of fluid in the affected area. As the reaction progresses in intensity the area of inflammation becomes "shunted off," as it were, from the rest of the organism. It develops its own pH, its own local circulation, and its own metabolism. Gessler [18] has demonstrated that the oxygen consumption and the metabolic rate are increased in an inflamed area. It is conceivable that the impairment of the local lymphatic and vascular circulation may promote in acutely injured tissues a state of relative anoxemia. With interference in normal oxidative reactions there is a possibility that an anaerobic type of glycolytic activity may result.* Lactic acid accumulates in abundance; and with the depletion of the alkali reserve in the area of injury, local acidosis becomes manifest. Owing either to a direct effect or else through the liberation of enzymes active at an acid pH, polymorphonuclear leukocytes degenerate. If the acidity increases beyond a pH of about 6.5, all types of leukocytes are injured and frank pus results.

* In view of the work of Crabtree referred to above, it is, however, possible that, as in the case of neoplasms, acutely inflamed tissues also may manifest some degree of aerobic glycolysis. This question is being investigated further.

The observations about to be described support further the original concept that in acute inflammation the cytological picture is a function of the hydrogen ion concentration.[1] The pH in turn depends on the rate of glycolytic activity and on the available local alkali reserve.*

## Experimental

Dogs were anesthetized by the intraperitoneal administration of an aqueous solution of nembutal (about 33 mg. per kilo weight). Pleural exudation was induced by the injection of 1.5 to 2 cc. of turpentine into the right chest. Subsequently a sample of the exudate was withdrawn daily over a period of several days by means of a Luer syringe containing about 1 cc. of 0.1 per cent heparin in saline. On withdrawing the sample several smears were made on slides. A part of the exudate was immediately transferred into a test tube under paraffin oil for pH determination. The rest of the exudate was centrifugalized under oil and the total $CO_2$ content of the cell-free material determined by the Van Slyke manometric method.[19] Samples of blood were obtained from the heart and centrifuged under oil. Total $CO_2$ determinations were likewise made on several samples of blood serum.

* The observations reported here and in a preceding communication have all been made on dogs. They have consistently revealed the fact that the pH seems to condition the cytological picture in inflammation. However, in some unpublished observations on the exudates of rabbits the correlation obtained between the cytological picture and the pH of the exudate was not as consistent as observed in dogs. Similar findings have recently been noted by Lurie (*Arch. Path.*, 1936, **22**, 272–288). It is interesting in this connection that in a review of intracellular digestion Opie (*Physiol. Rev.*, 1922, **2**, 552–584) cites the studies of various investigators who have either failed or found considerable difficulty in demonstrating proteolytic enzymes (readily demonstrable in dogs) in the leukocytes of rabbits.

However, preliminary studies, which are now being conducted in this laboratory and which are to be reported *in extenso* in the near future, indicate that direct studies of the degree of glycolysis (rather than resorting to pH measurements alone) in inflamed tissues of rabbits yield data that correlate quite adequately the cytological picture with the local disturbance in carbohydrate metabolism (see abstract in *Am. J. Path.*, 1936, **12**, 725–726).

In a recent careful study Clark and his co-workers (*Am. J. Anat.*, 1936, **59**, 123–173) point out the advisability of correlating the earlier pH studies of the senior author with living cells rather than resorting to fixed smears in order to rule out the possibility of mistaking modified polymorphonuclears for macrophages when the former cells become degenerated. Some observations were therefore made using the supravital technique on living cells. The results obtained indicated, as in fixed preparations, that the initial type of living cells in inflammatory exudates of dogs is the polymorphonuclear, whereas in the later stages of inflammation, when there is a corresponding rise in the hydrogen ion concentration, the preponderance of viable cells is of the macrophage type.

Measurements of the pH were always performed within a short time after withdrawing the sample of pleural exudate. As described in a previous communication,[1] the bicolor system of standards of Hastings and Sendroy [20] was employed in determining the hydrogen ion concentration.

The differential leukocyte counts were made from smears on slides. The cells were stained by the Wright method. In computing the percentage of polymorphonuclears and macrophages, cells were frequently encountered that were so degenerated as to render their identification difficult. These were not included in the final counts.

In the studies concerned with the intermediate carbohydrate metabolism of exudates as compared with that of blood, the samples

TABLE I

| Dog No. | Amount of turpentine injected | Duration of inflammation | Volume per cent of $CO_2$ content | | pH | | Cytology of exudate | |
|---|---|---|---|---|---|---|---|---|
| | | | Cell-free exudate | Blood serum | Exudate | Blood | Per cent of polymorphonuclears | Per cent of mononuclear phagocytes |
| | *cc.* | *hrs./mins.* | | | | | | |
| 6–2 | 2 | 20:30 | 40.9 | 45.4 | 7.4 | 7.5 | 86 | 14 |
| | | 43:45 | 38.6 | 41.1 | 7.15 | 7.5 | 85 | 15 |
| | | 68:15 | 29.0 | 45.0 | 6.5 | .. | 18 | 82 |
| | | 91:30 | 30.4 | 46.6 | 6.7 | 7.5 | 7 | 93 |

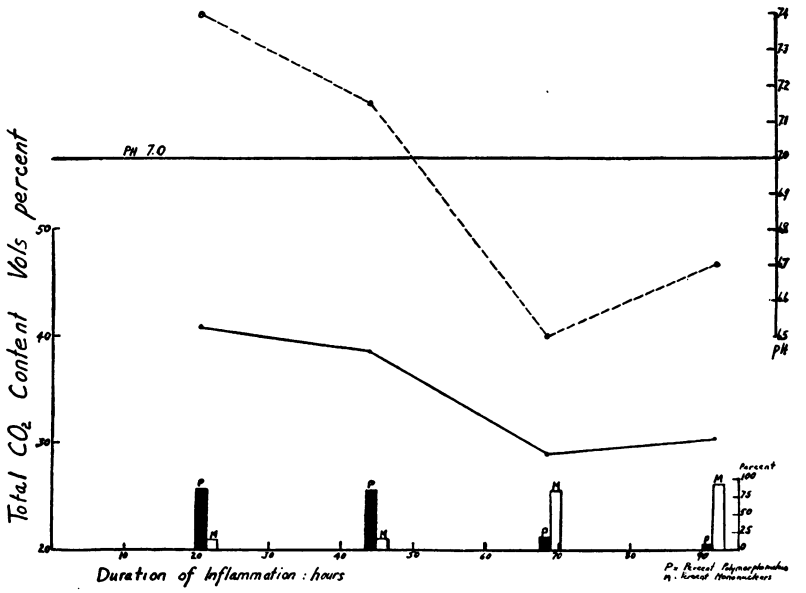
were obtained from the pleural cavity of dogs as described above. Lactic acid determinations were performed on exudates and whole blood by the method of Friedemann and Graeser.[21]* Blood and exudate sugar were determined by the technique of Folin.[23] Measurements of the hydrogen ion concentration and the cytological studies were likewise performed on these samples as described above.

In order to obtain a definite state of local acidosis in the inflamed area the reinjection of the irritant on the 3rd or 4th day was often found to be a convenient means of ensuring a significant fall in the pH.

* Stewart and his collaborators [22] claim that the ordinary methods for determination of lactic acid in muscle appear to yield results that are too high owing to interference by methyl glyoxal. Accordingly they advise removal of this substance by distillation prior to the treatment with copper-lime. Several determinations on exudates and blood after removal of methyl glyoxal yielded practically the same values for lactic acid as when this precautionary measure was omitted. To rule out the possibility that the lactic acid might primarily be derived from glycogen, several determinations for the presence of this substance in exudates were made. Glycogen could not be recovered to any measurable extent.

## The Depletion of the Alkali Reserve in Acute Inflammation

With the gradual development of local acidosis in an area of inflammation, the $CO_2$ content of the cell-free exudate correspondingly tends to fall. Analysis of blood serum, however, reveals no appreciable change in its $CO_2$ content. The point is illustrated in Table I which summarizes a type experiment.




Text-Fig. 1

The hydrogen ion concentration and the carbon dioxide capacity in relation to the cytological picture of a pleural exudate (Dog 6–2). Note that concomitantly with the fall in pH and alkali reserve there is a shift from a predominance of polymorphonuclear cells to a phase where mononuclear phagocytes constitute the chief element.

-------o------- pH of exudate

———·——— Total $CO_2$ content (volumes per cent) of cell-free exudate

▮ Percentage of polymorphonuclear cells

▯ Percentage of mononuclear phagocytes

This same data is graphically presented in Text-Fig. 1. The interrelation between pH, $CO_2$ content, and the cytological picture is quite apparent. With the fall in pH there is a concomitant reduction in the local alkali reserve, as measured by the $CO_2$ content. Parallel

with these changes in the acid-base equilibrium, the polymorphonuclear cells become displaced by mononuclear phagocytes. If the exudate remains alkaline throughout the period of the acute reaction, the $CO_2$ content likewise fails to be reduced. On the other hand, if a frankly purulent exudate develops with a pH of 6.5 or below, the $CO_2$ content may fall to about 8 volume per cent or less. The leukocytes in such types of viscous suppurating material are for the most part swollen, degenerated, and identified, if at all, only with the greatest of difficulty. The depletion in the alkali reserve adequately accounts for the production of an uncompensated acidosis at the site of inflammation.

## The Mechanism of Local Acidosis in Inflammation

Studies were undertaken to determine the nature of the acid produced in an area of inflammation. The experiments of Ito [24] had indicated the formation of d-lactic acid by the autolysis pus. Furthermore, as stated in the introductory portion of this paper, it is reasonable to suppose that with the impaired fluid exchange in an area of injury an anaerobic type of glycolytic respiration might prevail. This would manifest itself in a conversion of sugar into lactic acid. If the rate of glycolysis were to increase with no augmentation in the available alkali reserve, an uncompensated acidosis would necessarily result in the inflamed tissue. This seems to be precisely the course of events as indicated by the observations recorded in Table II. Five variable factors were studied in each experiment as follows: time relations, exudate and blood sugar, blood and exudate lactic acid, pH of blood and exudate, and the cytology of the exudate. All these variables in the case of the inflammatory exudate displayed what seemed to be a certain amount of interdependence. Fluctuation of one factor was inevitably followed by corresponding changes in the remaining variables. This is conveniently illustrated in Text-Fig. 2, in the case of Dog 9–2. For the first 3 days the pH of the exudate is alkaline and the polymorphonuclear cells predominate. The lactic acid concentration is relatively low; the level of the exudate sugar either equals or, as a rule, exceeds slightly that of the lactic acid (Table II). On the 3rd day the inflammatory reaction is accentuated by the reintroduction of the irritant. The level of the exudate sugar drops rapidly while the concentration of lactic acid correspondingly increases (Text-Fig. 2).

TABLE II

*The Relation of Lactic Acid Level to the Hydrogen Ion Concentration and to the Cytological Picture in Acute Inflammation*

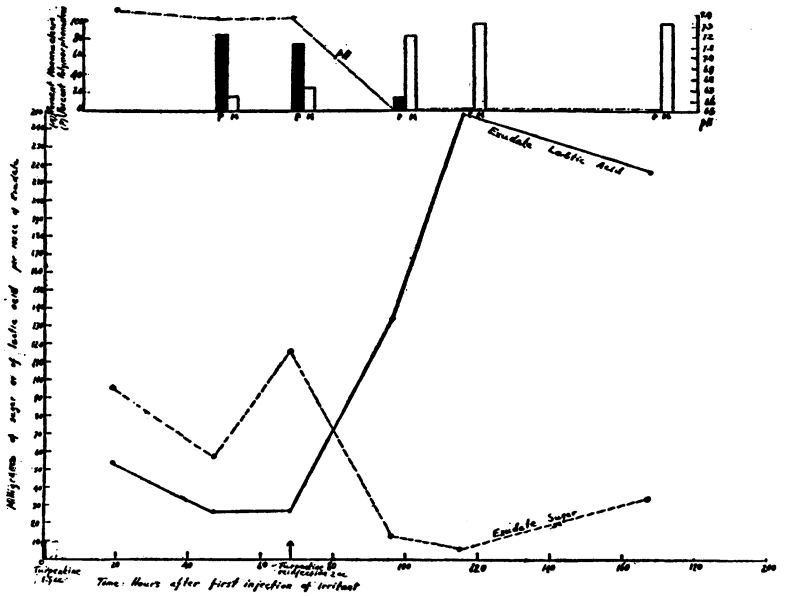
| Dog No. | Amount of turpentine injected | Total duration of inflammation | Sugar (mg. per 100 cc.) | | Lactic Acid (mg. per 100 cc.) | | pH | | Cytology of exudate | |
|---|---|---|---|---|---|---|---|---|---|---|
| | cc. | hrs./mins. | Blood | Exudate | Blood | Exudate | Blood | Exudate | Per cent of polymorphonuclears | Per cent of mononuclear phagocytes |
| 9–2 | 1.5 | 0:0 | 100.0 | .. | .. | .. | 7.50 | .. | .. | .. |
| | | 19:10 | 88.1 | 96.0 | 10.8 | 54.6 | 7.45 | 7.43 | Relatively acellular | |
| | | 47:10 | 111.9 | 57.9 | 13.0 | 27.0 | 7.48 | 7.33 | 85 | 15 |
| | | 68:0 | 145.5 | 116.8 | 16.0 | 27.8 | 7.48 | 7.33 | 74 | 26 |
| | 2.0 reinjected | | | | | | | | | (few |
| | | 96:15 | 81.2 | 13.9 | 21.9 | 135.8 | 7.43 | 6.5 | 15 | 85 cells) |
| | | 115:0 | 138.6 | 6.4 | 38.4 | 248.6 | 7.43 | 6.5 | 2 | 98 |
| | | 167:0 | 138.6 | 34.7 | 14.7 | 217.1 | 7.45 | 6.5 | 2 | 98 |
| 9–1 | 3.0 | 0:0 | 87.1 | .. | 18.1 | .. | 7.55 | .. | .. | .. |
| | | 19:0 | 76.2 | 52.5 | 14.3 | 59.9 | 7.45 | 7.30 | 90 | 10 |
| | | 47:05 | 82.2 | 48.0 | .. | .. | 7.43 | 7.43 | 66 | 34 |
| | | 67:45 | 102.0 | 89.1 | .. | .. | 7.43 | 7.28 | 82 | 18 |
| | 2.0 reinjected | | | | | | | | | |
| | | 95:50 | 81.2 | 12.6 | 10.3 | .. | 7.43 | 6.5 | Relatively acellular | |
| | | 114:40 | 86.1 | 9.9 | 11.3 | 147.8 | 7.43 | 6.5 | 8 (degenerated) | 92 |
| 8–1* | 3.0 | 0:0 | 108.9 | .. | 9.2 | .. | 7.33 | .. | .. | .. |
| | | 20:0 | 94.1 | 49.5 | 13.8 | 39.6 | 7.40 | 7.30 | Acellular | |
| | | 46:15 | 141.1 | 76.8 | 14.4 | 48.9 | .. | 7.17 | 84 | 16 |
| | | 68:15 | 108.9 | 42.6 | 27.4 | 69.7 | 7.45 | 7.33 | 90 | 10 |
| | | 95:45 | 141.6 | 46.0 | 24.5 | 58.0 | 7.48 | 7.33 | 88 | 12 |
| | 1.5 reinjected | | | | | | | | | |
| | | 115:0 | 81.2 | 12.9 | 10.0 | 110.5 | 7.48 | 6.83 | Relatively acellular | |
| | | 140:0 | .. | .. | .. | .. | .. | 6.6 | 4 | 96 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 8–5* | 3.5 | 0:0 | 78.2 | .. | 9.7 | .. | 7.48 | .. | .. | .. |
| | | 20:0 | .. | .. | 14.9 | 94.6 | 7.48 | 7.25 | Relatively acellular | |
| | | 47:45 | 90.1 | 18.8 | 11.5 | 101.1 | 7.50 | 6.93 | 44 | 56 |
| | | 67:45 | 106.0 | 38.1 | 9.2 | 71.8 | 7.58 | 7.10 | 86 | 14 |
| | | 95:45 | 118.8 | 58.2 | 8.8 | 55.4 | 7.50 | 7.20 | 84 | 16 |
| | | 115:30 | 92.1 | 78.2 | 10.3 | 57.8 | 7.45 | 7.10 | 67 (degenerated) | 33 |
| | 1.5 reinjected | 142:50 | 133.7 | 15.8 | 7.1 | 198.1 | 7.35 | 6.5 | 6 | 94 (degenerated) |
| 7–9* | 3.0 | 0:0 | 74.3 | .. | 16.7 | .. | 7.48 | .. | .. | .. |
| | | 24:10 | 64.4 | 21.8 | 32.6 | 88.5 | 7.38 | 7.15 | Acellular | |
| | | 72:20 | 95.0 | 40.6 | 21.2 | 91.4 | 7.40 | 6.88 | 28 | 72 |
| | | 94:55 | 114.8 | 58.9 | 9.9 | 78.8 | .. | 7.15 | 72 | 28 |
| 8–3** | 3.0 | 0:0 | 90.1 | .. | 9.2 | .. | 7.33 | .. | .. | .. |
| | | 27:20 | .. | 63.4 | .. | 52.1 | .. | 7.18 | Acellular | |
| | | 49:35 | .. | 28.7 | .. | 48.1 | .. | 7.30 | 92 | 8 |
| | | 76:55 | 118.8 | 58.4 | 14.7 | 66.0 | 7.48 | 7.05 | No count — preponderance of monuclears | |
| | | 96:35 | 121.8 | 72.8 | 15.3 | 37.2 | 7.48 | 7.30 | 70 | 30 |
| 8–4** | 3.5 | 0:0 | 92.1 | .. | 8.3 | .. | 7.48 | .. | .. | .. |
| | | 19:30 | .. | .. | 11.9 | 57.4 | 7.35 | 7.33 | Acellular | |
| | | 47:20 | 87.1 | 13.9 | 31.2 | 144.4 | 7.55 | 6.75 | Preponderance of mononuclears. Shrunken, vacuolated, degenerated leukocytes | |
| | | 67:20 | 103.0 | 23.3 | 11.8 | 132.0 | 7.55 | 6.6 | 24 (degenerated) | 76 |
| | | 95:30 | 150.5 | 15.1 | 13.4 | 134.9 | 7.50 | 6.70 | 22 (degenerated) | 78 |
| | No injection of acetamide for 3 days. (See footnote) | 115:05 | 117.8 | 10.1 | 15.5 | 128.9 | 7.50 | 6.80 | 12 | 88 |
| | | 215:45 | 93.1 | 93.1 | 15.4 | 52.1 | 7.60 | 7.50 | Leukocytes are degenerated and vacuolated 76 | 24 |

* Dogs Nos. 8–1, 8–5, and 7–9 received, in addition, daily injections of distilled water into the right pleural cavity.

** Dogs Nos. 8–3 and 8–4 received, in addition, daily injections of 1:1000 M mono-iodo-acetamide solution into the right pleural cavity.

The hydrogen ion concentration increases to a pH of 6.5. Polymorphonuclear leukocytes are found wanting or appear in small numbers as degenerated, swollen or distorted cells. The bulk of the cellular element consists of mononuclear phagocytes (*cf.* Text-Fig. 2, Fig. 2). Frequently on the 1st day following the injection of the




TEXT-FIG. 2

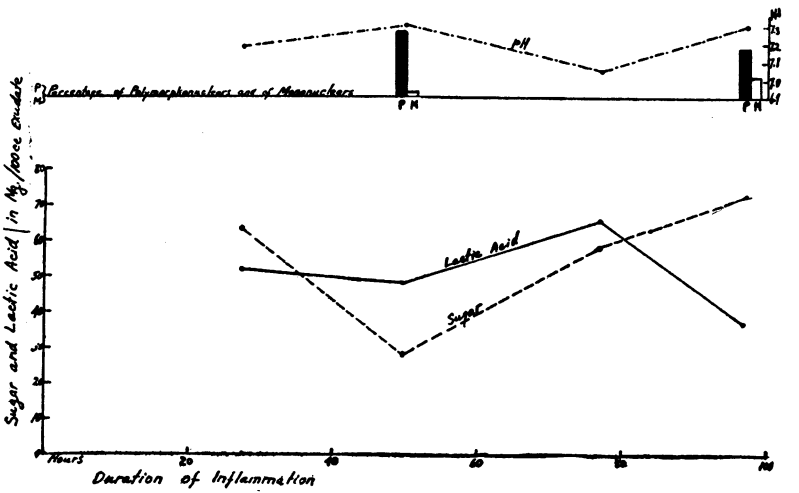
Correlation between the hydrogen ion concentration, the conversion of sugar to lactic acid, and the cytological picture of a pleural exudate (Dog 9–2). Note that with the fall in the level of the exudate sugar there is a rise in the concentration of lactic acid and the pH falls notably. This local acidosis is accompanied by a shift in cellular elements. The polymorphonuclear leukocytes are replaced by mononuclear phagocytes.

—·—·—·— pH
———·——— Lactic acid
———o——— Exudate sugar
▮ Percentage of polymorphonuclear leukocytes
▯ Percentage of mononuclear phagocytes

irritant, the exudate is found to be relatively acellular. Comparison of lactic acid and sugar figures in both blood and exudate reveals an interesting type of reciprocal relations (Table II). The blood sugar level is high compared to that in exudate, while the opposite is true in the case of lactic acid. It is conceivable that this state of affairs

may in part be referable to a higher degree of oxygen saturation in the circulating blood as compared to that in tissues.

The inflammatory reaction is not necessarily conducive to a state of local acidosis. The maintenance of an alkaline pH is, however, invariably associated with a predominance in the exudate of polymorphonuclear cells.[1] In such circumstances the abrupt conversion




TEXT-FIG. 3

Correlation between the hydrogen ion concentration, the exudate sugar, the lactic acid, and the cytological picture of a pleural exudate (Dog 8–3). Note that with the relatively low degree of glycolysis the pH is maintained at an alkaline range and the polymorphonuclear cells predominate throughout the course of the inflammatory reaction.

—·—·—·— pH
———·——— Lactic acid
———o——— Exudate sugar
▮ Percentage of polymorphonuclear leukocytes
[] Percentage of mononuclear phagocytes

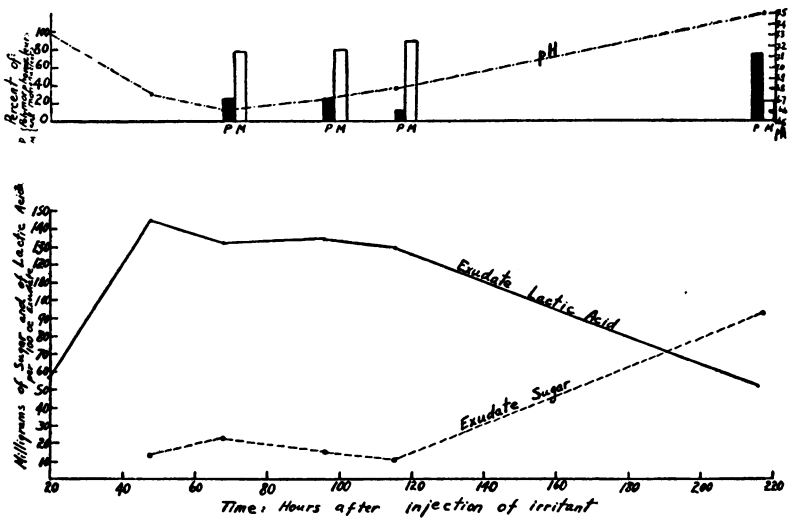
of sugar to lactic acid, as described above, fails to occur. This is well exemplified in Text-Fig. 3 (Dog 8–3, Table II). Within a period of 4 days the level of lactic acid failed to exceed 66 mg. per 100 cc. of exudate, and the pH never fell below 7.0. This would suggest that in an inflamed area when the alkali reserve is depleted it is primarily the rate of formation of lactic acid that determines the ultimate degree of acidity.

The studies of Lundsgaard [25] indicated that a muscle poisoned with iodoacetic acid fails to produce lactic acid during the contractile phase. The introduction of iodoacetic acid into the inflamed pleural cavity of dogs was found to be impracticable owing apparently to the toxic action of the drug. Therefore we substituted a daily injection of 10 cc. of a 0.001 M solution of mono-iodo-acetamide into the chest cavity that had previously been inoculated with turpentine.* It was believed that such a procedure might inhibit the glycolytic process and consequently maintain a predominance of polymorphonuclear leukocytes in the inflamed area. The control animals received repeated superimposed injections of distilled water instead of the iodo-acetamide solution (*cf.* Dogs 8–5, 7–9, 8–3, 8–1, and 8–4, Table II). It is clear from the data that iodo-acetamide failed *in vivo* to prevent the usual course of the inflammatory reaction leading to an increase in lactic acid. It is to be noted that in a previous communication one of us had attempted to alter *in vivo* the pH of the area of inflammation by the repeated introduction of phosphate buffers.[1] The effort ended in failure; this served as an additional example of the fact that *in vitro* reactions are not easily reproduced in the living organism. Evidently more information on the mechanism of tissue buffers would be desirable before attempting to modify experimentally the acid-base equilibrium in the extracapillary spaces. In the case of one dog (No. 8–4), the repeated injections of the acetamide apparently even hastened the development of local acidosis (*cf.* Table II and Text-Fig. 4). The lactic acid rose rapidly at the expense of the sugar level and the pH correspondingly fell. There was a preponderance of mononuclear phagocytes. After about 1 week the daily injections of iodo-acetamide were discontinued for a period of 3 days. At that time the pH was found to be alkaline, the exudate sugar had risen with a corresponding drop in lactic acid, and the cellular content consisted now primarily of polymorphonuclear leukocytes (Text-Fig. 4). The observations on this particular dog suggest that with cessation of daily injections of mono-iodo-acetamide reparative processes of vascular organization were favored; thus the sprouting of new capillaries permitted wider opportunity for oxygenating the inflamed tissue. This state of affairs probably tended to decrease the glycolytic rate and thereby the hydrogen ion concentration.

* This substance was obtained through the courtesy of Dr. G. Pincus.

## Discussion

The foregoing observations, in addition to evidence obtained in a previous communication,[1] indicate that the cytological picture in an area of acute inflammation is apparently conditioned by the local pH, which in turn is determined by the rate of lactic acid formation




Text-Fig. 4

The hydrogen ion concentration, the lactic acid, the exudate sugar, and the cytological picture of a pleural exudate (Dog 8–4). This animal received in addition daily injections of a solution of 0.001 M mono-iodo-acetamide into the affected pleural cavity for about a week. Note the failure in inhibiting the sharp initial glycolysis with the concomitant rise in the hydrogen ion concentration. The mononuclear phagocytes form the predominating cellular element. On discontinuing the injections of iodo-acetamide the pH rose and the glycolytic activity was found to be considerably diminished. At this stage the polymorphonuclear cells assumed the ascendancy.

—·—·—·— pH
———·——— Lactic acid
- - - -o- - - - Exudate sugar
▮ Percentage of polymorphonuclear leukocytes
▯ Percentage of mononuclear phagocytes

and the depletion of the alkali reserve at the site of injury. With progress in the intensity of the inflammatory reaction there is a tendency for increased glycolytic activity as revealed by a rise in lactic acid formation. This is accompanied by a fall in the carbon

dioxide capacity and the pH correspondingly drops. A true lactic acid acidosis results at the site of inflammation. The polymorphonuclear leukocytes apparently survive only when the pH is alkaline (Text-Fig. 1, Fig. 1). As the pH falls below 6.7 or thereabouts, all types of leukocytes tend to be injured and frank pus develops. It is conceivable that the mechanism of suppuration is in part referable to the development of this local acidosis resulting from an increased glycolysis. The high degree of acidity may well act as a toxic factor on leukocytes. The studies of Evans [26] indicate that these cells are sensitive to the action of acids. It is also possible that the development of an acid pH favors the action of proteolytic enzymes released from leukocytes (*e.g.* pepsin and cathepsin) as well as that of other tissue autolytic enzymes.[27,28] Studies on the mechanism of suppuration are now in progress and will form the subject of a separate future communication.

The present observations indicate the important rôle of disturbed carbohydrate metabolism in an area of injury. In view of the available evidence, it is likely that differences in histological manifestations of various infectious lesions may be referable to disturbances in the intermediary carbohydrate metabolism of the affected tissue. It is conceivable that the intensity and severity of an inflammatory reaction is primarily referable to its capacity for bringing about the incomplete oxidation of carbohydrates. In this connection it is interesting to note that within a given interval of time *Staphylococcus aureus* induces a lesion characterized by a greater glycolytic activity than does *Streptococcus hemolyticus*. Previous work had demonstrated that the localization of staphylococci in contrast to the invasiveness of hemolytic streptococci was primarily referable to the greater necrotizing capacity of the former microorganism.[29,30] The implications of such a concept in regard to possibly providing an adequate explanation for the well known fulminating character of infectious lesions in the diabetic condition are sufficiently obvious without warranting any additional comment.

Several problems of theoretical interest arise as a result of the present series of experiments, suggesting some similarity between the chemical dynamics involved in muscular contraction and in the development of the inflammatory reaction. For instance, it is well known that hexosephosphate represents an intermediary product in the breakdown of glycogen to lactic acid. Cori and Cori [31] have

demonstrated that tetanic stimulation of muscle yields hexosephosphate. Under anaerobic conditions the removal of this compound is affected by glycolysis for it is accompanied by an equivalent increase in lactic acid and by the liberation of inorganic phosphate. The writers have accordingly studied the inorganic phosphate content of cell-free exudates and compared it with that in samples of blood serum. In the initial stages of inflammation the level of phosphates in exudates is slightly higher than in serum. After several days, however, when the inflammatory reaction has progressed in intensity and the exudate assumes a purulent character, the phosphate content is found to be several times more elevated in the cell-free exudate than in the serum. Concomitantly, as pointed out in this communication, the lactic acid concentration is found to be considerably augmented. This would strongly suggest a similarity between the changes in carbohydrate metabolism during muscular activity and those occurring in an acutely inflamed area where there presumably develops a state of relative anoxemia owing to impaired local vascular and lymphatic circulation.[17]

Rubel [32] has recently studied the relation between glycolysis and proteolysis in tissues. The observations of this investigator indicate that an increase in glycolytic activity of tissue is accompanied by an accumulation of amino and of non-protein nitrogen, whereas inhibition of glycolytic activity is followed by a diminution of proteolytic processes. It may be noted here that parallel determinations in exudate and blood serum have invariably revealed a higher concentration of amino acid nitrogen in the exudate than in the blood serum.[33] This difference in level was particularly striking in the later stages of the inflammatory reaction at a time when the lactic acid concentration was elevated. Furthermore, the concentration of total protein nitrogen was found to be lower in the exudate than in the corresponding samples of blood serum. This reciprocal relation between amino acids and total protein nitrogen in exudate and serum suggests active proteolytic processes at the site of inflammation, particularly at the time when the rate of glycolysis is considerably enhanced.

Hegnauer, Fenn and Cobb [34] have recently pointed out that in muscles of frogs with notable increase in potassium concentration, irritability is completely held in abeyance and simultaneously lactic acid production begins to be enhanced. In a recent study from this

laboratory [35] it has been shown that in an inflammatory exudate the concentration of potassium is about twice as high as in serum, even in the earliest phase of the reaction. Whether this high concentration of potassium in inflammation is in any way related to the increased glycolytic rate remains to be determined.

In brief, various questions on the chemistry of inflammation suggested by the present line of investigation are being studied further in an endeavor to obtain a clear notion of the relation of the intermediary products of carbohydrate metabolism to differences in the morphological manifestation of a variety of infectious lesions. These studies will form the subject of separate reports in the future.

## Summary and Conclusions

With the development of an acute inflammatory reaction the carbon dioxide capacity of the cell-free exudate progressively diminishes. This is correlated with an increase in the hydrogen ion concentration and by a concomitant shift in the cellular composition from a polymorphonuclear to a mononuclear phagocytic phase. When the pH drops below 6.7 or 6.5, most of the leukocytes appear to be injured and frank suppuration ensues.

An inflammatory exudate manifests greater glycolytic activity than blood, as indicated by the higher level of exudate lactic acid and a correspondingly lower concentration of exudate sugar.

The rate of glycolysis increases as the inflammatory reaction progresses in intensity. Within several days, particularly if the reaction has been intensified by reinoculating the irritant, the concentration of lactic acid is considerably augmented and the result is a localized lactic acid acidosis.

The evidences indicate that the mechanism of local acidosis in inflammation is therefore primarily referable to an increase in the rate of glycolysis and a consequent depletion of the alkali reserve. With the increase in the hydrogen ion concentration to a pH below 7.0 polymorphonuclear leukocytes seem unable to survive and the predominating infiltrating cell is the mononuclear phagocyte. A maintenance of an alkaline pH resulting from relatively diminished glycolytic activity is accompanied by a preponderance of polymorphonuclear leukocytes with no subsequent shift in the cellular constituents of the exudate.

The available evidence indicates that the cytological picture in an

area of acute inflammation appears to be conditioned by the local pH which in turn depends on the rate of glycolysis and the depletion of alkali reserve. The significance and implications of local disturbances in carbohydrate metabolism in determining the severity of an acutely inflamed area have been discussed.

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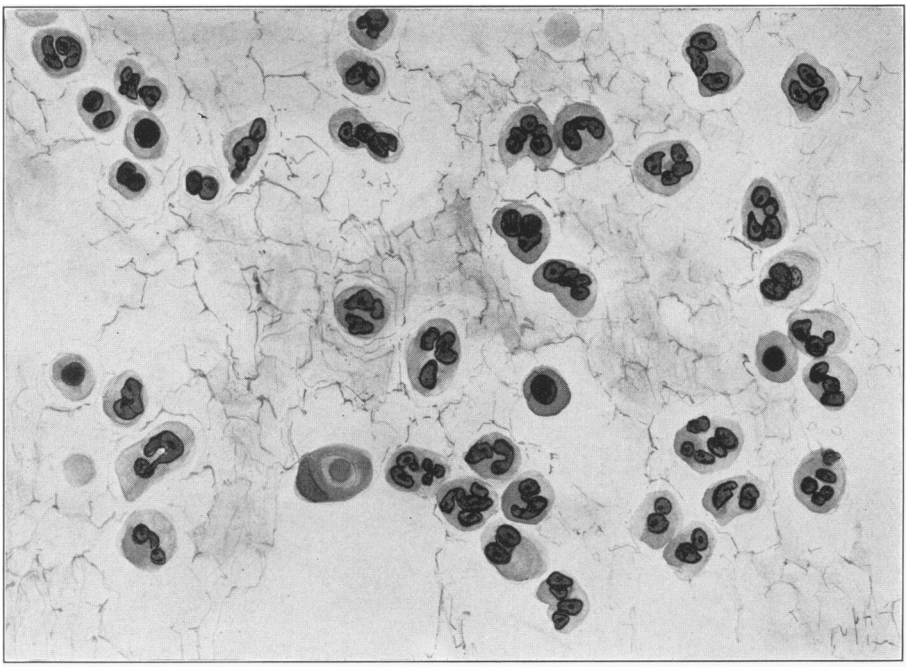
## DESCRIPTION OF PLATE

### PLATE 4

FIG. 1. Camera lucida drawing of inflammatory reaction induced by the injection of turpentine in Dog 5–0. Reaction is of 24 hours duration. Note preponderance of polymorphonuclear cells; pH of exudate is 7.45. × 1300.

FIG. 2. Camera lucida drawing of inflammatory reaction induced by the intrapleural injection of turpentine in Dog 9–2; reaction is of 1 weeks duration. Note preponderance of mononuclear phagocytes; pH of exudate is approximately 6.5. × 1300.



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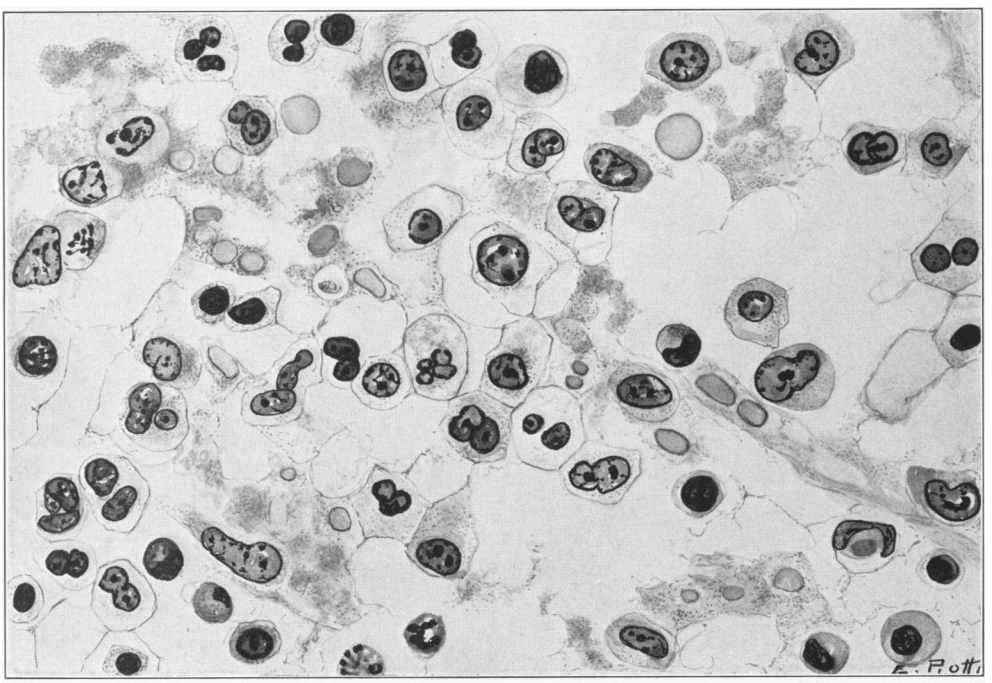


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