# SOME OBSERVATIONS CONCERNING THE EFFECT OF ACUTE, SEVERE HEMORRHAGE DIURNAL VARIATION AND SEASONAL TEMPERATURES ON THE BLOOD LACTIC ACID LEVEL OF THE DOG

by

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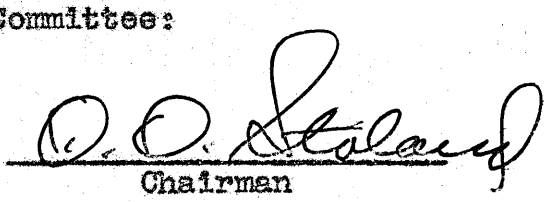

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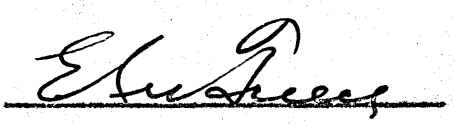

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## STATEMENT OF THE PROBLEM

The concentration of lactic acid in the circulating blood of an animal has assumed considerable importance in the study of: (1) the normal metabolic processes of the animal organism, (2) the detection of deranged metabolism, and, (3) the therapeutic measures employed to combat disease.

The particular purpose of this study was to investigate some of the factors which may affect the level of lactic acid in the blood of the normal, healthy, resting dog. These factors are:

(1) The delayed effect of hemorrhage on the lactic acid level in the blood

(2) The effect of diurnal variation

(3) The effect of seasonal temperature variation.

Furthermore since there is no generally accepted norm for blood lactic acid it was thought that a large series of determinations might furnish information which would be helpful in the attempt to establish some central figure as a norm for the species.

## REVIEW OF EARLY WORK ON LACTIC ACID

The first record of the isolation of lactic acid is found in C.W. Scheele's publications (1780). In his work on the whey from soured milk, he was able to isolate a thick, syrupy liquid which he called the acid of milk.

Berzelius (1848) appears to have been the first to recover lactic acid from animal tissues. His material was secured from freshly killed animals.

Little advance with respect to the significance of lactic acid in muscle was made until the research of Fletcher and Hopkins (1907). These investigators proved that the lactic acid formed in amphibian muscle tissue during activity can be disposed of by the mechanisms existing within the muscle itself, provided the oxygen supply to the muscle is maintained.

Meyerhof (1920) working on excised frog muscle found that glycogen in the muscle was the source of the lactic acid. His work showed, in addition, that three-fourths of the lactic acid removed during the oxidative phase was synthesized in the muscle to glycogen. On the basis of this and further work by Meyerhof and others it was assumed that the energy liberated by the formation of lactic acid from glycogen was used directly for muscular contraction.

This view of the role of lactic acid was largely held until Lundsgaard (1930) demonstrated the contraction of muscle, even though the formation of lactic acid was pre-

vented by the use of mono-iodo-acetic acid. Lundsgaard (1930) advanced the hypothesis, now usually held, at least for the activity of isolated muscle, that lactic acid formation releases energy used in the resynthesis of the compounds utilized for direct energization of the muscle fiber. In addition, the earlier observation that oxidation of some of the lactic acid furnishes energy for the re-synthesis of glycogen is quite generally held.

The existence of lactic acid in blood was indicated by Berzelius, according to C. Enderlein (1843) yet he (Enderlein) was unable to confirm this observation of Berzelius.

Although many attempts failed -- Enderlein (1843), Salomon (1878), Jerusalem (1908) --other investigators, Gaglio (1886), Berlinerblau (1887), Frey (1895), Ryffel (1909), Fries (1911), Barcroft et al (1915), Clausen (1922), Barr, Himwich and Green (1923) and others found lactic acid present in blood in amounts varying, under more or less normal conditions, from 6.0 mgm. per cent (Fries, (1911) to 32.1 mgm. per cent (Clausen, 1922).

Long (1924) was the first to observe conditions of strict muscular rest in his normal experimental subjects. He required his patients to lie down for one-half hour before the sample of blood was withdrawn. Glycolysis was avoided by the use of sodium fluoride as recommended by Evans (1922). In this series of five determinations, using four different subjects, he obtained from venous blood

lactic acid values ranging from 17.5-35.6 mgm. per cent. The method of Clausen (1922) was employed for determination of blood lactate.

It is, at the present time, accepted that lactic acid is a normal constituent of the blood according to Book, Dill, and Edwards, (1932), even though its source is not entirely accounted for.

# METHOD OF ANALYSIS

## PREPARATION OF THE BLOOD

The blood was drawn into a glass syringe which had been rinsed with saturated sodium oxalate solution. After ejection of the solution, enough dry sodium oxalate was added to prevent coagulation of the blood. Sodium oxalate has been shown to retard glycolysis (Macleod, 1913) and not to interfere in the determination of lactic acid content.

Blood was secured from peripheral veins in the legs, although occasionally the external jugular was used. When the required amount of blood had been obtained, it was emptied into a glass beaker and five cubic centimeters were measured by an Ostwald pipette and delivered into a 250 c.c. Erlenmeyer flask.

Protein removal was secured by using the Folin-Wu tungstic acid method (1919) as modified by Haden (1923). Into the Erlenmeyer flask, 40 c.c. of $\frac{N}{12}$ sulfuric acid were delivered slowly with constant agitation of the contents of the flask. 5 c.c. of 10% sodium tungstate solution were delivered into the diluted, hemolysed blood. The sample was allowed to stand five minutes or longer to allow complete precipitation of the proteins. To remove the protein material, the sample was filtered.

10 c.c. of the filtrate were pipetted off and delivered into a 50 c.c. Erlenmeyer flask. Van Slyke's (1917) method for removal of carbohydrates was employed in which 1 c.c.

of 25% Cu $SO_4$ was added along with sufficient dry calcium hydroxide to make the solution alkaline. The flask was shaken frequently during at least a thirty minute period. The contents were then centrifuged, stoppered and placed in the refrigerator. Ronzoni and Wallen-Lawrence (1927) have shown that standing at refrigerator temperature the lactic acid content is unaffected during a period of at least seventy-two hours. A series of experiments by us (1940) confirmed this and, in addition, showed no change in lactic acid values during a period of two weeks in the refrigerator.

## ISOLATION AND TITRATION OF LACTIC ACID

The lactic acid content of the blood samples was determined by the Friedemann, Cotonio and Shaffer (1927) and Friedemann and Kendall (1929) modifications of the Von Fürth-Charnass (1910) iodometric procedure as described by Peters and Van Slyke (1932).

Heating lactic acid with manganese dioxide suspension oxidizes the lactic acid to acetaldehyde:

$$CH_3 . CH(OH) . COOH + O = CH_2 . CHO + CO_2 + H_2O.$$

The acetaldehyde is removed from the oxidation flask and driven into sodium bisulfite by employing a current of air previously washed through sodium bisulfite solution. This preliminary washing secures the removal of all bisulfite binding materials in the air current. With the bisulfite in the collecting flask, the aldehyde forms an addition

compound:

$$CH_3.CHO + NaHSO_3 = CH_3.CHO.NaHSO_3$$

The bisulfite-aldehyde compound does not react with iodine, but the unbound bisulfite does:

$$NaHSO_3 + 2I + H_2O = NaHSO_4 + 2HI.$$

The unbound bisulfite is removed by Ripper's (1900) method of adding 0.1N. iodine, using 1 c.c. starch solution as indicator, until a slight excess of iodine is present. The excess iodine is removed by addition of one drop of 0.1N sodium thiosulfate. 0.002N iodine is then added until a clearly discernible blue color appears in the colorless solution.

The combined bisulfite-aldehyde is set free by the addition of a small amount of sodium bicarbonate in either dry form or solution. The bisulfite set free is then titrated with 0.002N iodine. This modification of Ripper's method was introduced by Clausen (1922), who thus converted the original method of determination by difference into a direct titration of bound bi-sulfite.

## CALCULATION

One cubic centimeter of 1N iodine is equivalent to 0.5 millimole or 45 milligrams of lactic acid. To obtain the number of millimoles of lactic acid in a liter the following formula is used:

$$\text{Millimoles of lactic acid per liter} = \frac{500\ N(A - B)}{V}$$

Where N is the normality of the iodine solution used to titrate the sodium bisulfite bound by aldehyde, A is the cubic centimeters of iodine used, B is the cubic centimeters of iodine used in a blank analysis, and V is the cubic centimeters of blood used in the sample.

When N is 0.002N and the sample represents 5/11 cubic centimeter of blood, the equation becomes:

$$\text{Millimoles lactic acid per liter blood} = \frac{(500)\ (0.002)(A - B)}{5/11}$$

$$= 2.2(A - B)$$

or,

expressing the value in milligrams per 100 c.c. blood,

$$\text{Milligrams lactic acid per 100 c.c. blood} = \frac{(45)(0.002)(A - B)(100)}{5/11}$$

$$= 19.8(A - B).$$

# DELAYED HYPERLACTACIDEMIA FOLLOWING SEVERE HEMORRHAGE

# INTRODUCTION

This study is concerned with the investigation of the lactic acid level in the blood of otherwise normal dogs following an acute, severe hemorrhage. More particularly, the intent was to determine the lactic acid content of the blood during the later stages of the recovery from the severe hemorrhage. No samples were taken until the end of the first twenty-four hour period following the blood loss.

Other workers have shown that significant blood lactic acid changes occur during the first twenty-four hour period. Riegel (1927) observed in dogs a quick rise in concentration of lactic acid, followed by a gradual return to normal. During removal of thirty-one per cent of the calculated blood volume in a typical case from Riegel's series, the lactic acid content had increased from 18 mgm. per cent to 35.4 mgm. per cent. One hour after cessation of blood withdrawal, the lactic acid content was 19.5 mgm. per cent, or back to the normal figure.

The magnitude and duration of the lactic acid increase, in Riegel's opinion, depend upon the extent of the hemorrhage. However, in all her experiments the lactic acid content had returned to the normal level before the end of the first twenty-four hour period following the blood loss.

In addition, this investigator's results in two experiments indicated a secondary rise which occurred some thirty to forty-eight hours following the blood removal, and, perhaps,

represented a sequel to the increase during the first twenty-four hour period. However, the author's technique and discussion of results were of such nature as to fail to indicate if this response was a part of the usual recovery period following extensive blood loss or whether it was some inconstant factor such as excessive weakening of the animal following a particularly severe hemorrhage. Riegel says, "It is interesting to note that in every case (except where hemorrhage terminated in death) on the day following the hemorrhage the concentration of lactic acid in blood is again normal---."[1]

Fuss (1934), in an experiment on two dogs noted that the lactic acid content of the blood rose and then fell back to the normal resting value by the end of the first twenty-four hour period following severe hemorrhage. He failed to follow the recovery period further.

It was the purpose of the present investigation to ascertain if the secondary, delayed increase in blood lactic acid is a constant part of the recovery period following an acute, severe hemorrhage.

[1] Cecilia Riegel, "Formation of lactic acid in the body after severe hemorrhage." Journal of Biological Chemistry, 74: 133, 1927.

## PROCEDURE

Six dogs were selected, of which three were males and three were females. Each was confined in an individual cage throughout the experiment. The same attendant cared for the dogs during the course of the experiment. Feeding was done twice daily - at eleven A.M. and again at four P.M. The temperature of the room in which the cages were kept ranged from fifteen to twenty degrees Centigrade. Two dogs, one male and one female, were included as controls.

All blood samples were drawn at the same hour of the day - between seven P.M. and nine P.M. Peripheral blood from the leg veins only was used for lactic acid determination. The blood was drawn into a glass syringe containing enough dry sodium oxalate to prevent coagulation. Exact 5 c.c. amounts were drawn into Ostwald pipettes and delivered into diluting flasks. In every case, the puncture, withdrawal, dilution with N/12 sulfuric acid and precipitation of the proteins with ten per cent sodium tungstate were completed within a five minute period, thus avoiding glycolysis.

The blood filtrates, after removal of carbohydrates, were placed in stoppered centrifuge tubes and kept in the refrigerator. The lactic acid content of each was determined within a week.

The dogs were well trained and lay quietly on the table

as the assistant held them in position.

Hemorrhage was achieved by direct cardiac puncture without anesthetizing the dog. About thirty per cent of the calculated blood volume was removed during the one hemorrhage.

On the day preceding the hemorrhage and on the same day as the hemorrhage, but just prior to the blood loss, a sample was taken from each dog. These lactic acid values were taken as the normal, resting value for each dog. The recovery period after hemorrhage was studied by taking samples at the same hour each day following the blood loss until the end of the fifth day. Thereafter a sample was taken every two days until the end of the ninth day.

## RESULTS

In Table I a complete record of the data is presented. Examination of the two lactic acid values obtained from each dog before hemorrhage reveals that there is considerable variation even in these samples from normal, resting subjects. Dogs 1, 2, and 5 show higher lactic acid values in the second sample than in the first. On the other hand, dogs 3, 4, and 6 show a decrease when their second sample is compared to their first. There was only one dog -#3- which shows approximately similar values on the two days, although as noted above, this dog's lactic acid decreased, yet the decrease was only 2% on the second day as compared to the first.

After withdrawal of blood from each dog varying from 25% to 32% of the calculated blood volume, no samples were drawn for twenty-four hours in order to avoid further complication due to added blood loss. Riegel (1927) and Fuss (1934) have shown that there is a rapid increase during hemorrhage, or shortly thereafter, followed by a rather prolonged decline, but that in all their experiments the blood lactic acid level had returned to normal by the end of the first twenty-four hour period following the hemorrhage. The determinations on our series of dogs showed low twenty-four hour values in all dogs except number 6, in which dog the highest peak for that animal of the series was reached.

On the second day after hemorrhage, decided increases in lactic acid were found in all dogs except number 6 whose lactic acid had, by this time decreased to the normal level.

The third day, post-hemorrhage, gave values slightly higher than those of the second day in every dog except number 1, whose sample was lost during titration. The lactic acid values obtained on the fourth day were generally lower than any previous results, either normal or after hemorrhage. The fifth day gave values generally higher than on the previous day and in one case, number 4, the highest value for that animal and also for the series was obtained.

From this point, samples were drawn every other day. The next samples, drawn seven days after the hemorrhage

gave values which, in most cases, were below the "normals". In fact, in all but dogs 2 and 3 the values were the lowest of the whole series. On the ninth day, the lactic acid concentrations had again risen to about the "normal" value, although in one dog, number 3, the concentration was quite high.

## DISCUSSION

The behavior of lactic acid in dog's blood follows a general pattern during the recovery period after hemorrhage. This can be seen by comparing Figures 1 and 2. In Figure 1, while there is considerable variation, yet, in general, it can be noted that the variation before hemorrhage is at random. Twenty-four hours after hemorrhage the lactic acid levels are, with two exceptions, at the resting level for any given dog.

In five of the six dogs, the lactic acid has sharply increased by the end of forty-eight hours. Approximately the same high level is present at the end of seventy-two hours. Then, without exception, lower values are found at the end of ninty-six hours. Some of the ninty-six-hour values are lower than the pre-hemorrhage levels.

From the ninty-sixth hour to the end of the experiment--where the majority of the levels approached the pre-hemorrage resting values--the lactic acid variations occur in unison. Some variations are of greater magnitude than others,

however. Throughout the duration of the recovery period there are periodic fluctuations of considerable extent. Figure 2 shows the trend of the group as a whole and it can be seen that, in general, each dog reflects the performance of the whole group.

In some way, then, the random behavior of the lactic acid level has been modified by the hemorrhage so that it displays a periodic nature.

It is obviously not totally a response to a deficient oxygen supply to the tissues, even though this may cause a hyper-lactacidemia as has been shown by Araki (1891) and many others since. It is difficult to see how the tissues could be adequately supplied with oxygen at the end of twenty-four hours and be inadequately supplied in the forty-eighth hour, although the animal was in a state of rest during both periods.

Doubtless, a hyper-lactacidemia, such as Riegel (1927) described, during or immediately following hemorrhage is best accounted for on a basis of oxygen deficiency. Gesell et al. (1930a) have shown in anesthetized, operated animals that such a hyper-lactacidemia is found following a reduction in oxygen content in the respired air. Similarly after hemorrhage Gesell and his colleagues (1930b) obtained high lactic acid values in the blood. These experiments were of short duration and on animals under conditions not normal. Hence it may be questionable to attempt a direct transposition of results.

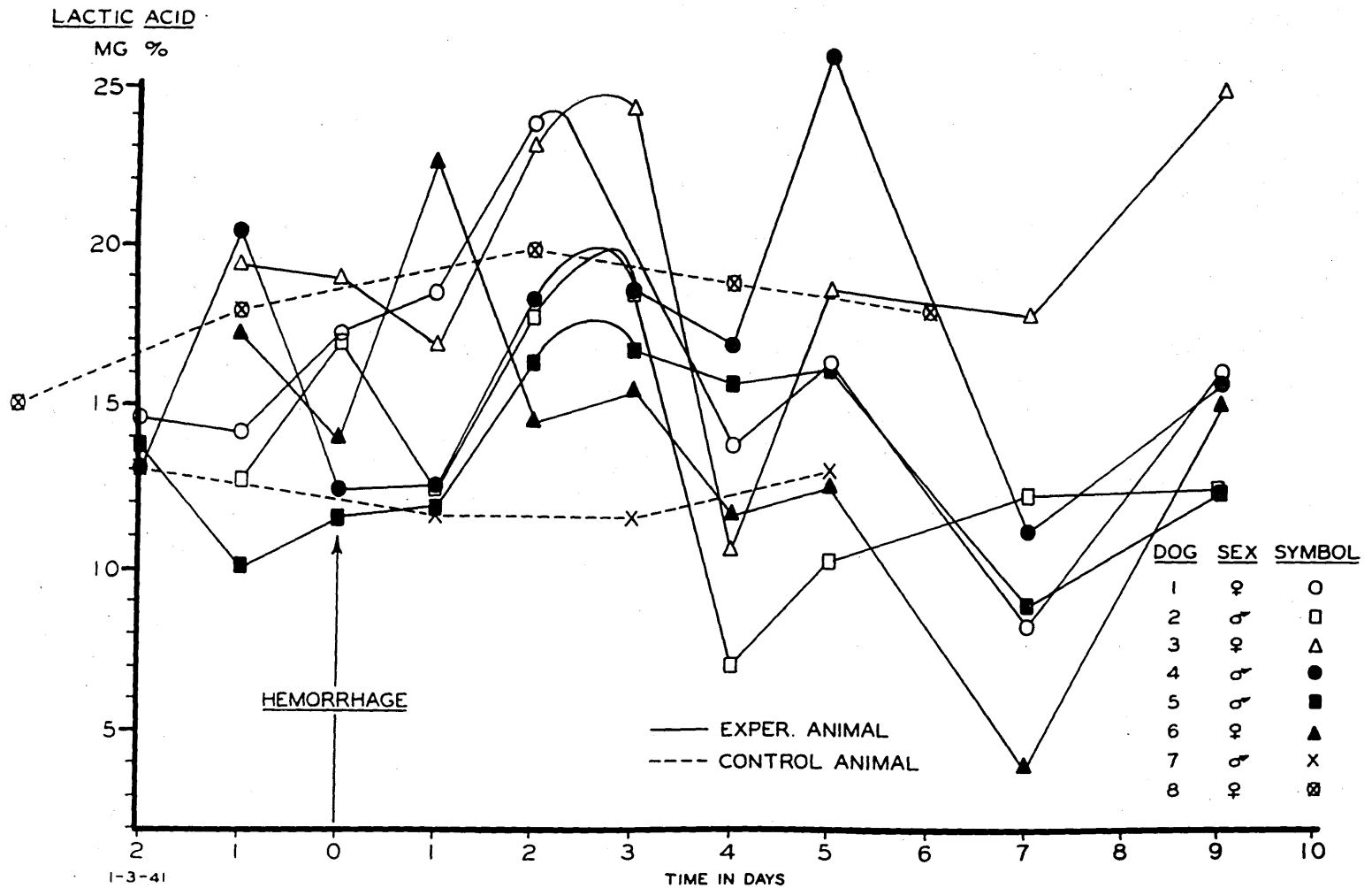


**FIG. 1** EFFECT OF SEVERE HEMORRHAGE ON BLOOD LACTIC ACID—NORMAL DOGS

NOTE LACTIC ACID LEVEL IS GENERALLY LOW ON TWENTY-FOURTH HOUR AFTER HEMORRHAGE. ALSO GENERALLY HIGH VALUE ON SECOND AND THIRD DAYS AFTER HEMORRHAGE.

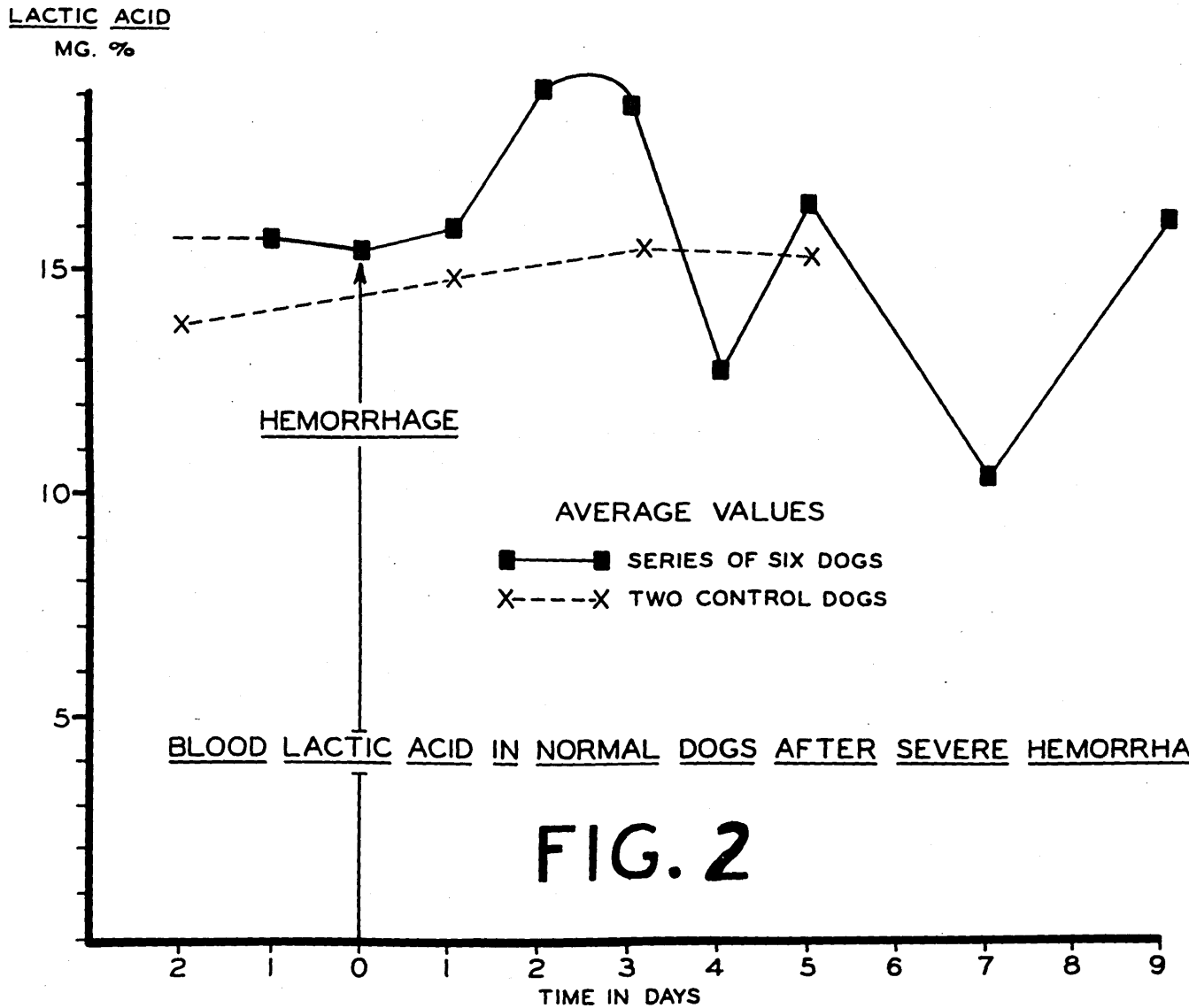
LACTIC ACID
MG. %
15
10
5
HEMORRHAGE
AVERAGE VALUES
SERIES OF SIX DOGS
TWO CONTROL DOGS
BLOOD LACTIC ACID IN NORMAL DOGS AFTER SEVERE HEMORRHAGE
2 1 0 1 2 3 4 5 6 7 8 9
TIME IN DAYS

FIG. 2

Particularly is this true in view of the experiments of Cook and Hurst (1933). These investigators, using healthy human subjects, found that the lactic acid content of the blood during rest was the same as during a period of light to moderate exercise. Even when their subjects walked at a rate of three m.p.h., no increase in blood lactic acid could be found in venous blood immediately draining the active muscle groups.

Jervell (1928) in studying the lactic acid concentration in the blood of anemic patients was unable to demonstrate any consistent relationship between hemoglobin percentage or red cell counts and lactic acid. Jervell concluded that deficient oxygenation is seldom, if ever, serious enough in anemic patients to prevent the combustion of the amounts of lactic acid normally produced.

Bock, Dill and Edwards (1932) failed to find any significant change in blood lactic acid of normal men following reduction of inspired oxygen to nine per cent, ingestion of sodium bicarbonate or ammonium chloride; or taking moderate exercise. These investigators demonstrated a change in pH and alkaline reserve in some of the above procedures, but they could demonstrate no consistent relationship between hydrogen ion concentration and lactic acid changes in the blood of their subjects.

Summing up their results, in the light of work done by other investigators, Bock, Dill and Edwards (1932) remark:

"If moderate muscular exercise in untrained men can be executed without appreciable change in the level of blood lactic acid, it appears improbable that the lactic acid formed during resting states has its origin only in muscle metabolism. Taking into account the relative stability of lactic acid concentration during rest, it seems better not to attempt a specific explanation but to go no further than to regard this phenomenon as a part of the general process as a whole. We suggest, for example, that it may be a split product in the oxidation of carbohydrate, mobilized for the maintenance of general body needs. Much work with reference to glucose oxidation must be done before the question can be settled. Of the lactic acid present in the blood of a resting subject a small portion may come from muscle activity, the rest presumably from the activity of the central nervous system, various glandular activities, etc. It seems logical to suppose that the ability to reconvert lactic acid to its precursor may vary greatly at the seat of its formation, just as the rate of utilization of oxygen may vary from organ to organ in the body. The problem remains for study along lines differing from those suggested in the past."

It is our opinion, too, that the level of blood lactic acid in the resting subject must be accounted for in a way differing from any suggested previously. Therefore, we venture such a suggestion.

A possible explanation of the lactic acid response is afforded by linking the lactic acid level of the subject, whether in ordinary rest or in rest following severe hemorrhage, with the showers of leucocytes which are liberated into the blood stream.

The evidence, at present, is only suggestive since there is little in the literature concerning extended observations of the leucocyte count as related to other physiological variables. The observations which have been reported, although fragmentary, indicate that there may be

some relation between the presence of leucocytes and lactic acid concentration in blood of resting subjects.

This is not to be construed as denying the well established fact that lactic acid varies in the blood under conditions of physical stress. Our purpose is to call attention to some similar aspects of leucocyte changes and lactic acid.

Drinker, Drinker and Kreutzmann (1918) in one of the few extended experiments on the cellular content of the blood of dogs following large hemorrhage show that the liberation of leucocytes into the peripheral blood follows a pattern which shows many similarities to the lactic acid variations in our experiments.

A typical series of leucocyte counts following a large hemorrhage on three of the dogs from Drinker's (1918) experiments are given in Table 2.

The data obtained by Drinker, Drinker and Kreutzman (1918) are shown in tabular form in Table 2. In Figures 3 and 4 the data are plotted. The data from our hemorrhage and lactic acid studies are also plotted. In this way the striking increases and decreases in both leucocyte counts and lactic acid concentration can be compared.

It is easily seen that there is no direct relation between any two given lactic acid and leucocyte curves. Such agreement is not to be expected since the two variables were determined on different dogs at widely separated times. The experimental conditions were different, since leucocyte

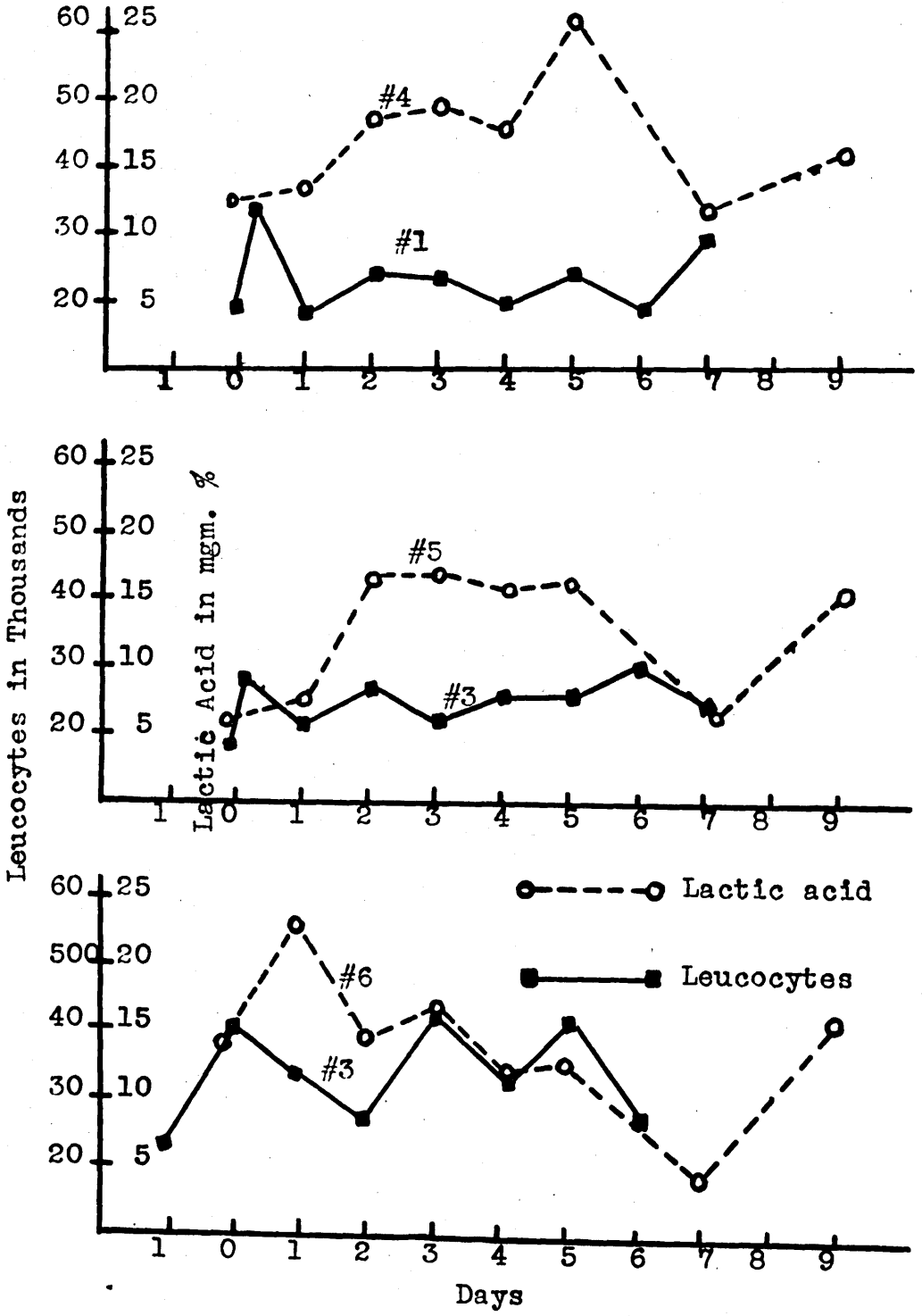


Fig. 3. Blood lactic acid and leucocytes in dogs following severe hemorrhage. Leucocyte values from Drinker, Drinker and Kreutzman, Jour. Exp. Med., 27, 383-397, 1918.

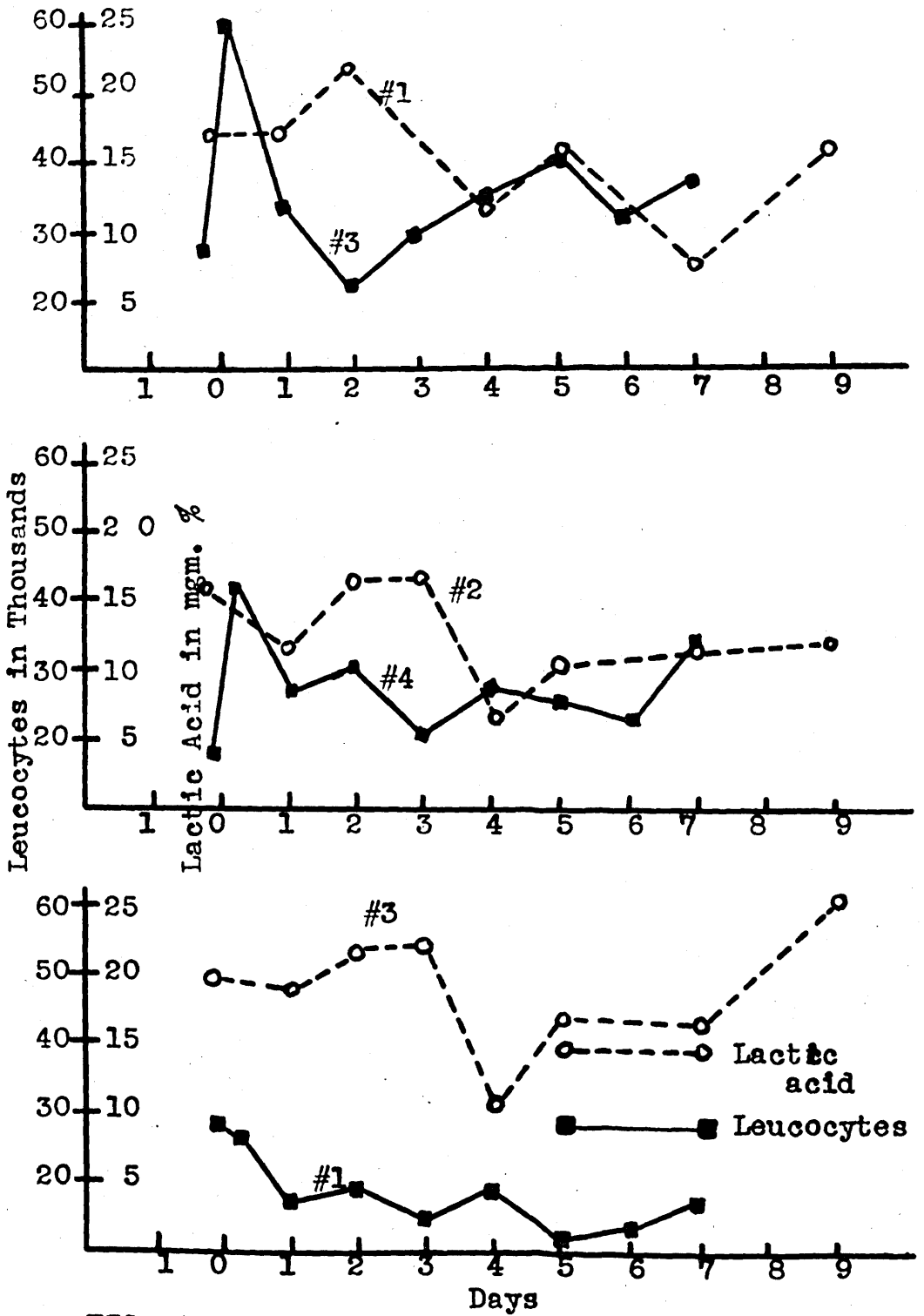


FIG. 4. Blood la ctic acid and leucocytes in dogs following severe hemorrhage. Leucocyte values from Drinker, Drinker and Kreutzman, Jour. Exp. Med., 27, 383-397, 1918.

counts were obtained during a study of the effect of repeated hemorrhages and saline infusions under anesthesia, while our lactic acid values were taken following one severe hemorrhage on normal, resting dogs.

The significant point to be observed concerning the two variables is that the direction and rate of variation are similar. They simply show that, when an animal is subjected to a severe blood loss, the blood picture with respect to either variable can vary drastically from day to day in the recovery period. At this point, all that can be said is that future work may show a relationship between the lactic acid content of the blood and the leucocyte count.

## SUMMARY

1. The blood lactic acid level during recovery from severe, acute hemorrhage consisting of 25-32% of total blood volume in three male and three female dogs was studied by taking daily samples of peripheral blood. Each daily sample was drawn at the same hour, the subjects being in complete rest.

2. After hemorrhage high lactic acid values were found in five dogs on the forty-eighth hour and were still present on the seventy-second hour. One dog varied from the general response, in that lactic acid peak was observed on the twenty-fourth hour.

3. From the third day until the end of the ninth day, when the study ended, the lactic acid curve shows well defined peaks and troughs, indicating a rhythmic variation as compared to the random blood lactic acid variation of the normal resting subject.

4. No significant difference according to sex was observed.

5. Evidence is given which suggests that the variation in leucocytes may account for some of the above phenomena.

TABLE I

Daily blood lactic acid values in dogs before and after severe hemorrhage

| Dog | 1 | 2 | 3 | 4 | 5 | 6 | Ave. |
|---|---|---|---|---|---|---|---|
| Sex | Female | Male | Female | Male | Male | Female | |
| Wt. in Kgm. | 4.5 | 6.4 | 4 | 6.4 | 8 | 8 | |
| | | | Lactic Acid in Mgm. Per Cent | | | | |
| Date | | | BEFORE HEMORRHAGE | | | | |
| 1-4-41 | 14.2 | 12.8 | 19.4 | 20.4 | 10.1 | 17.3 | 15.7 |
| 1-5-41 | 17.3 | 17.2 | 19.0 | 12.6 | 11.7 | 14.1 | 15.3 |
| | | | AFTER HEMORRHAGE | | | | |
| | | | Per Cent of Total Blood Volume Removed | | | | |
| 1-5-41 | (30) | (30) | (32) | (28) | (25) | (28) | |
| 1-6-41 | 18.6 | 12.5 | 17.0 | 12.7 | 12.3 | 22.6 | 15.9 |
| 1-7-41 | 23.7 | 17.8 | 23.0 | 18.4 | 16.4 | 14.6 | 19.0 |
| 1-8-41 | -- | 18.6 | 24.2 | 18.6 | 16.8 | 15.6 | 18.7 |
| 1-9-41 | 13.9 | 7.1 | 10.7 | 17.0 | 15.8 | 11.9 | 12.7 |
| 1-10-41 | 16.4 | 10.3 | 18.6 | 25.7 | 16.2 | 12.7 | 16.6 |
| 1-12-41 | 8.3 | 12.3 | 17.9 | 11.3 | 7.9 | 4.0 | 10.3 |
| 1-14-41 | 16.2 | 12.5 | 25.0 | 15.8 | 12.5 | 15.2 | 16.2 |

TABLE II

Total white cell count in dogs after severe hemorrhage*

| Time | Number of Dog 1 | 1 | 4 | 3 | 3 | 3 |
|---|---|---|---|---|---|---|
| | Total White Blood Cell Counts | | | | | |
| | BEFORE HEMORRHAGE | | | | | |
| 1 hr. | 18,000 | 28,000 | 13,000 | 26,500 | 22,000 | 18,000 |
| | AFTER HEMORRHAGE | | | | | |
| 1 hr. | 33,500 | 26,000 | 43,000 | 60,000 | 41,000 | 28,000 |
| 1 day | 14,500 | 17,000 | 27,000 | 33,000 | 33,500 | 21,500 |
| 2 days | 23,500 | 18,500 | 30,000 | 22,500 | 26,500 | 27,500 |
| 3 days | 23,000 | 13,500 | 20,000 | 29,000 | 41,500 | 21,500 |
| 4 days | 17,000 | 18,000 | 27,000 | 34,500 | 32,000 | 23,000 |
| 5 days | 24,000 | 11,000 | 25,000 | 39,000 | 42,000 | 25,000 |
| 6 days | 15,000 | 13,000 | 22,000 | 31,000 | 26,000 | 30,000 |
| 7 days | 29,500 | 16,000 | 34,000 | 36,000 | 31,000 | 24,000 |

* This data is taken from C. K. Drinker, K. R. Drinker, and H. A. Kreutzmann. Journal Exp. Med. 27: 383-397, 1918.

# DIURNAL VARIATION IN BLOOD LACTIC ACID

# INTRODUCTION

In Table III are given some representative blood lactic acid values taken from the literature and other sources. It is interesting to note the extreme variation in values, not only from author to author but also from low to high values given by any particular author. The percentage variation, in all but one case, is at least 100%.

Such variations as the above are not the only ones which ought to be considered when evaluating data dealing with blood lactic acid concentration in relation to physiological and experimental manipulations. The literature contains little reference to any extended investigation of the blood lactic acid concentration with respect to: (1) the variation which might be expected in an unanesthetized, resting subject from time to time, and, (2) the regularity of such variation, i.e., diurnal variation. Authors may regard changes in blood lactic acid concentration of the magnitude of 15-20 per cent as being significant. This would hardly seem justified if one may expect a 100% variation in the normal, resting subject, as would be indicated from the literature.

In this investigation an attempt has been made to ascertain if there is a diurnal variation in blood lactic acid in the normal, resting dog. In addition a study has been made of the variation from dog to dog and of the possibility of using some central figure as a norm in re-

TABLE III

Typical normal blood lactic acid values from various authors

Normal Blood Lactic Acid Values

Man

| Author | Number of Determinations | Values in Mgm. % Low | High | Ave. |
|---|---|---|---|---|
| Clausen (1922) | 3 | 21.8 | 32.1 | 28.2 |
| Barr, Himwich and Green (1923) | 6 | 14.0 | 25.2 | 19.2 |
| Long (1924) | -- | 10.0 | 20.0 | -- |
| Schultze (1926) | -- | 9.0 | 13.0 | 11.0 |

Dog

| | | | | |
|---|---|---|---|---|
| Gaglio (1886) | -- | 17.0 | 157.0 | -- |
| Collazo and Lewicki (1925) | 3 | 19.6 | 134.0 | 58.5 |
| Houget (1933) | 13 | 10.0 | 69.0 | 32.0 |
| Binet and Klukowski (1933) | 66 | -- | -- | 28.0 |
| Edwards, Brouha Johnson (1938) | -- | 5.4 | 14.6 | -- |
| Ivy, Crandall (unpublished) | 135 | 6.0 | 25.0 | 12.9 |
| Swan (unpublished) | 234 | 1.98 | 31.28 | 10.77 |

ferring to the expected lactic acid concentration.

## PROCEDURE

Three differing plans of attack were employed in the experiments to determine the existence of fluctuations in blood lactic acid of a diurnal character.

### Procedure I

In this procedure, the dogs were designated as Group A. They were selected from the general dog pen and allowed to rest for forty-five minutes before the first sample was drawn. Food was withheld from the animal during the twenty-four hour period of sampling. Every precaution was taken to insure conditions of complete rest.

Males and females were used on alternate test periods, so that there should be no preponderance toward males or females. In so far as possible, a different dog was used for each test period. However, it was found necessary to use dog #7 for three test periods.

This series of experiments was continued for a twelve month period. Each test period included twenty-four samples, distributed an hour apart throughout the twenty-four hour period. Each twenty-four hour run was made once a month on approximately corresponding days.

### Procedure II

The dogs were designated as Group B. Seven animals -- three females and four males--were included in this class.

One dog died after two months which necessitated an immediate replacement, thus bringing the total number to seven, instead of the intended six.

The dogs in this group were kept in individual cages throughout the whole period of thirteen months. Feeding was done at 4:00 P.M. each day, except on the days when test runs were made at which time no food was given.

A twenty-four hour test run was made only once during any given month. Each dog was bled just four times during a twenty-four hour period. The four samples were spaced six hours apart. The time of bleeding of the six dogs was staggered so that a sample was obtained every hour due to appropriate alternation of the animals. Each dog was started on a bleeding schedule in which the samples were taken six hours apart. This same schedule was followed each month from December, 1939 through July 1940. Thus, every month, when a test run was made, a given dog furnished blood for the same four hours as in every other month in this period.

The samples for January, 1940 were omitted because the time for analysis of the blood filtrates was so far removed from the time of drawing the blood, that it was decided not to include them in the series. For the rest of the period, there are twenty-four runs recorded for consecutive months.

## Procedure III

The dogs here included are referred to as Group C. Six dogs -- the same animals which comprised Group B, except the dog which died -- were subjected to a period of experimentation for the six months from August, 1940 through January, 1941.

These animals were given the same care and treatment as in Procedure II except in one respect, namely, the manner of rotation of sampling time. Each dog was bled every six hours as in Procedure II, but month by month each dog's bleeding schedule was altered so that at the end of the six-month period each dog had contributed one sample for each hour of the twenty-four hour period.

## RESULTS

The data indicate that there are two distinct periods of high lactic acid concentration during a given twenty-four hour test. Examination of Tables V-VIII, inclusive, show that one high tide occurs about noon and the other about twelve hours later, i.e., near midnight.

These two tides are shown in all three groups of dogs. In Group A, as shown in Table XVI and in Figure 5, the average hourly values near mid-day and mid-night are around three mgm. per cent higher than the low values a few hours previous.

The mid-day rise in Group A begins near 10 o'clock, reaches a peak at 12 noon and then subsides to below the mean value for the group by 4 P.M. The mid-night rise is quite pronounced, but it lacks the well sustained character of the mid-day increase. Its peak is a little higher than the mid-day peak, yet it exhibits a somewhat fluctuating nature, since the 12 mid-night value is lower than the 11 P.M. or the 1 A.M. values.

One transient rise occurs at about 6 P.M. It appears and disappears quickly, although its magnitude is almost equal to either of the two previously mentioned high tides.

Well defined troughs occur around 8 P.M. and 5 A.M. to 8 A.M. These are of short duration and soon give way to the strong upward movements previously described.

Referring to Tables V-VIII, the individual performances are seen to bear considerable resemblance to the variations mentioned above.

A consideration of Group B, which includes the dogs sampled every six hours throughout the twenty-four hour test period, shows much the same kind of response as was obtained in Group A. Referring to Table XVI and to Figure 6, it is seen that, in general, this curve exhibits the same form as the curve for Group A. As one would expect, however, there are greater fluctuations from hour to hour due to the fact that individual peculiararities exert a disproportionately large influence. This arises from the

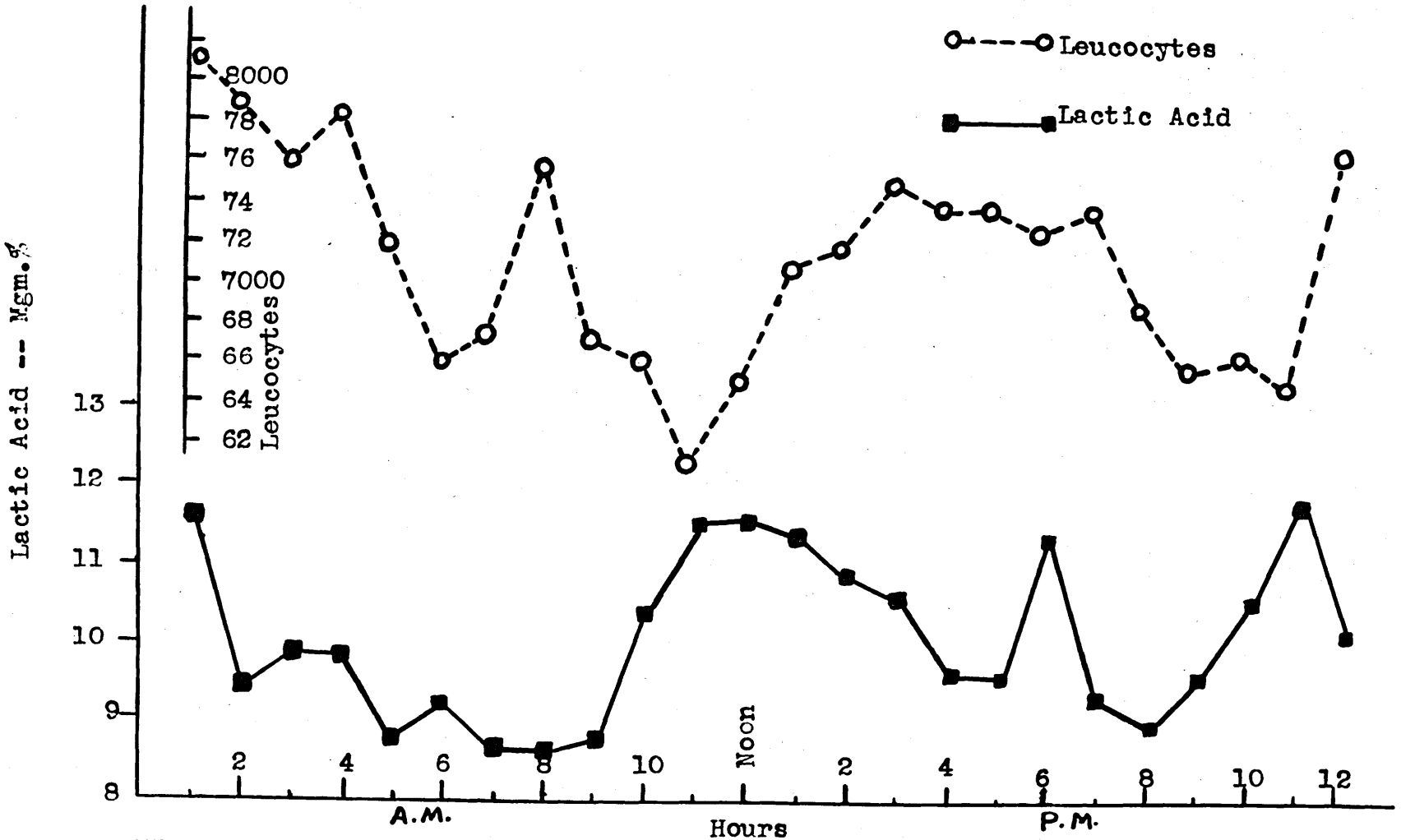


FIG. 5 Hourly variation of lactic acid in dog's blood compared to Hourly variation of leucocytes in men. Data for W.B.C. from Shaw, A.F.B., Jour. Path. and Bact., 1927, 30, 1-19.

fact that each dog was sampled on the same hours during each test period throughout the seven months.

The curve exhibits a well defined increase which begins shortly after 12 noon and reaches its peak at 4 P.M. Another high tide is seen at 1 A.M. This increase is of quite brief duration, since it lasts for only an hour. The increases at 7 A.M. and 7 P.M. are of some magnitude but are probably best interpreted as reflecting individual peculiarities rather than diurnal variations.

The trough, occuring from 2 A.M. to 6 A.M., is well defined. A second trough at 9 P.M. exhibits a more transient nature.

By following through any given individual's performance, it can be seen that such an individual exhibits, in general, a behavior which simulates the composite performance. That is, a dog may have consistently high lactic acid values, but, if so, it is seen that his highest values lie in the high tide phase of the curve.

Group C -- Where the dogs were alternated so that each dog contributed a sample for each of the twenty-four hours -- shows well defined peaks around noon (See Figure 7) and at midnight. In this regard, the curve conforms quite well to the results obtained from Groups A and B.

In Figure 7 the late evening rise occupying the four-hour period from 5 P.M. to 9 P.M. is observed to be exceedingly well defined. A late afternoon rise of lesser magnitude can be observed in each of the preceding curves.

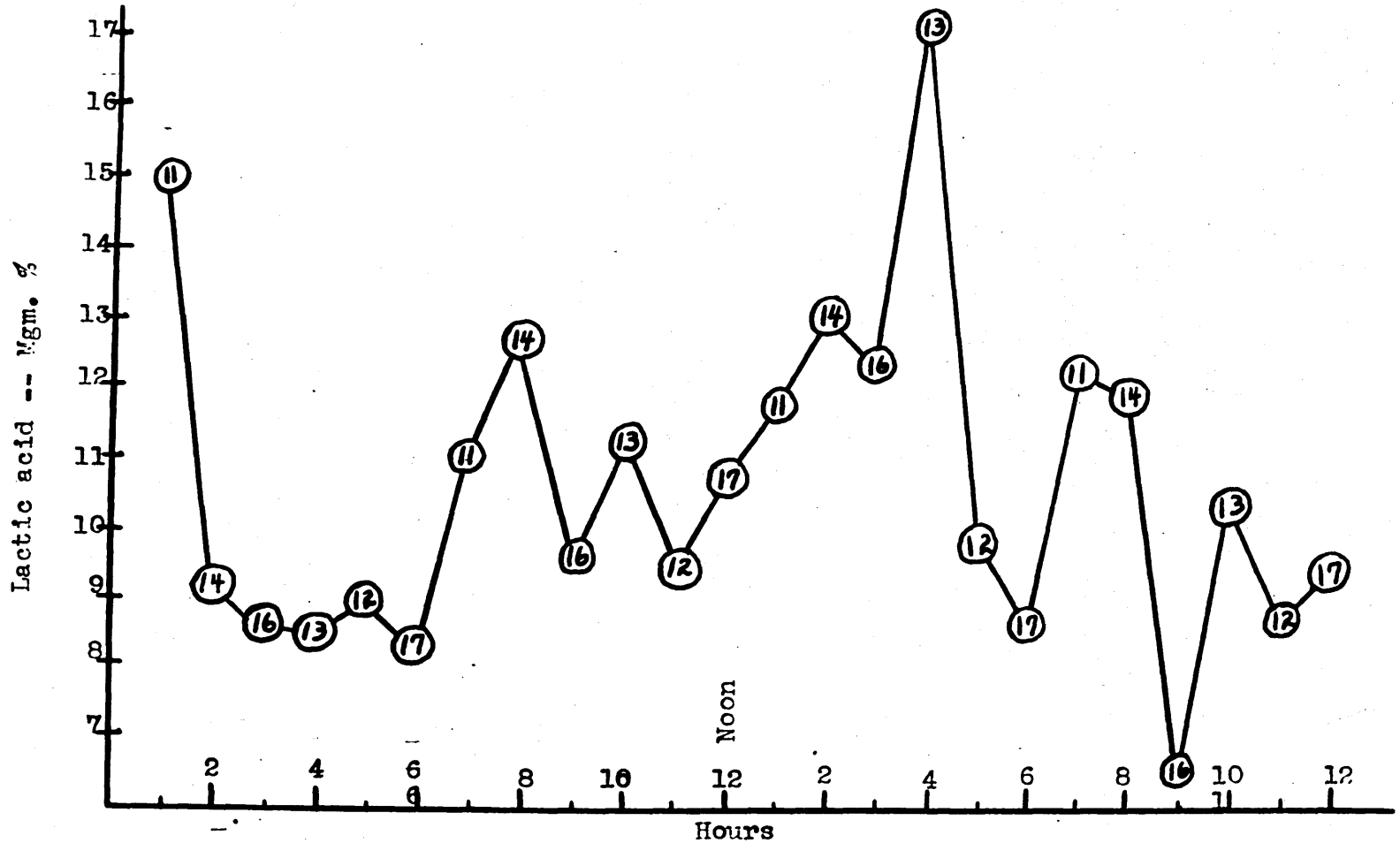


**Figure 6.** Diurnal va riation in blood lactic a cid in dogs. These dogs made up Group B. Points are numbered to correspond with number of a particular dog. See text for details.

Although these increments vary in duration and magnitude, the time of their appearance is of such uniformity as to be of significant import. Moreover, it is believed that the experimental conditions to which the Group C dogs were subjected were of such nature as to bring out clearly any true, significant change in lactic acid concentration.

## DISCUSSION

The results indicate clearly a blood lactic acid concentration in the dog that shifts within rather wide limits under conditions which achieve a complete state of rest in the animal. This range in our series varied from 1.98 to 38.80 mgm. per cent. In the dog showing the widest variation -- dog #6 -- the limits were 3.17 to 38.80 mgm. per cent. The dog showing the narrowest range -- dog #9 -- varied from 11.28 to 22.97 mgm. per cent.

According to the above figures, the difference from the lowest value of the entire series to the highest value represents a twenty-fold increase. In the case of the dog with the widest range, a twelve-fold increase is evident. The dog with the narrowest range exhibits an increase of 100%. These variations represent changes of unexpected magnitude, in light of which all work based on a comparison with resting lactic acid values may well be re-examined.

The diurnal variations, which embrace significant elevations in the twenty-four hour study near mid-day, mid-mid-night and near 6 P.M., are well enough defined to warrant

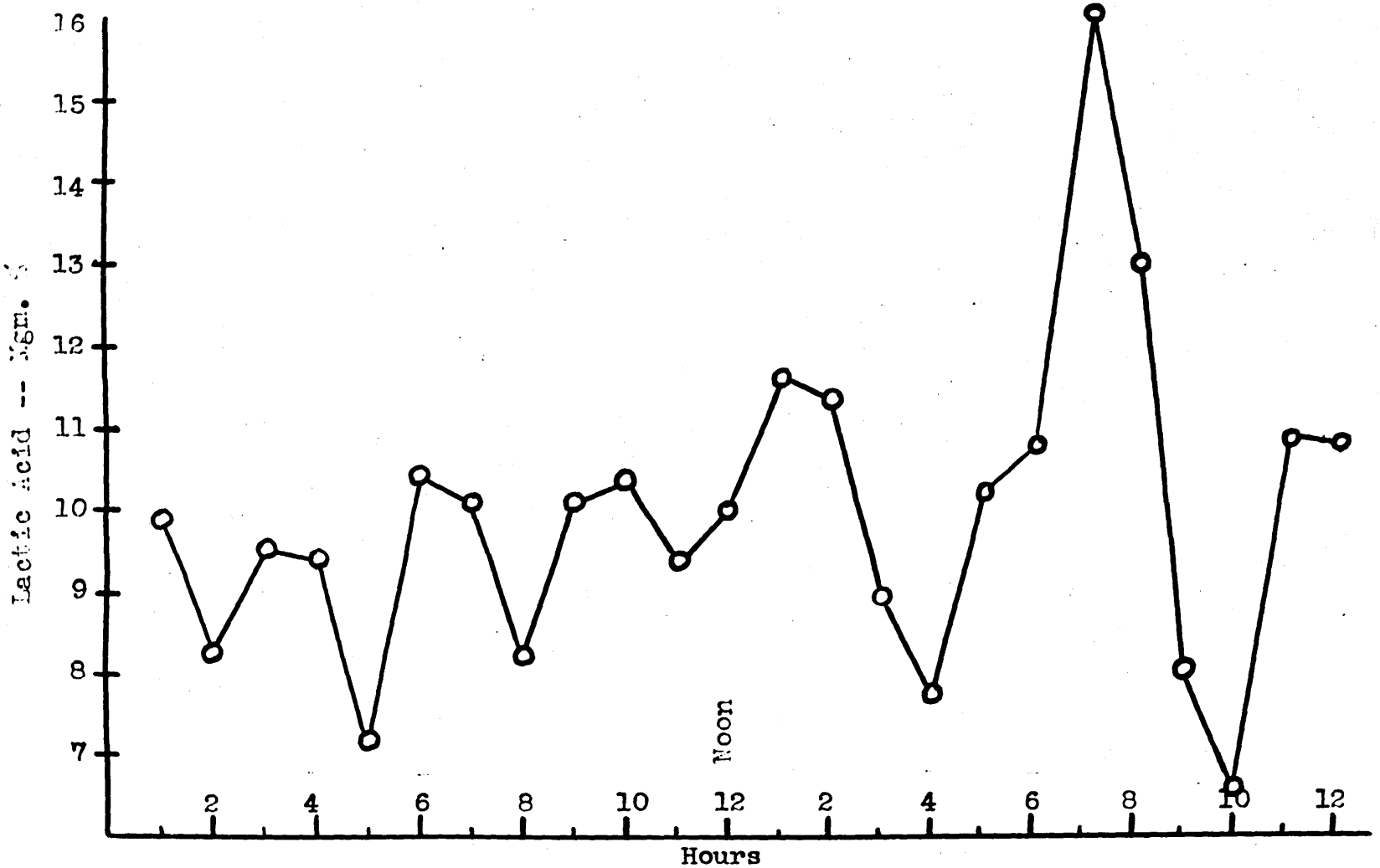


Figure 7. Diurnal variation of blood lactic acid in dogs of Group C.

close inspection. The mid-day rise begins at, or slightly before, noon in all of the three groups and reaches a crest some two or three hours later. This rise is of longer duration than those occurring at other times in the twenty-four hour period. The mid-night swell is of shorter duration, but it may rise to a higher peak than the mid-day crest. The late afternoon peak is usually of quite short duration, although it may occupy some four hours as in the case of Group C. These three crests constitute significant diurnal variations in the concentration of a constituent of the blood which previously has been considered as being present in rather non-fluctuating amounts while the organism is in a state of rest (Bock, Dill and Edwards, 1932).

Lactic acid in the blood of the resting organism has been considered the result of acid production by contracting muscles and its subsequent diffusion into the blood stream. Recent experimental work on man by Bock, Dill and Edwards (1932) and Cook and Hurst (1933) fails to account for more than a part of the resting blood lactate as due to such course. Bock, Dill and Edwards (1932) suggest that resting blood lactic acid "may be a split product of carbohydrate mobilized for the maintenance of the general metabolism of the body." This generalization is so vague that it offers little aid in explaining the facts.

A comparison of the behavior of blood lactic acid

with the behavior of other constituents of the blood under conditions of complete rest reveals the interesting fact that the only constituent in the blood which exhibits diurnal variations of comparable frequency and magnitude is the white blood corpuscle. Further study of the concentration of these structural elements suggests a striking similarity to the concentration of lactic acid.

One careful twenty-four hour study on the diurnal variation of leucocytes is that of Shaw (1927) in which he followed the leucocyte changes in four normal human subjects by performing white cell counts every hour throughout a twenty-four hour period. Sabin et al. (1925) studied the normal rhythm of the white cells in resting man covering the daylight hours of 9 A.M. to 4 P.M. only. Tschishikow (1927) studied the so-called digestion leucocytosis in dogs and included a curve showing the rhythm of the leucocytes in resting, fasted dogs during the period from 8:20 A.M. to 9:30 P.M. Zirm and Bauermeister (1933) more recently followed the leucocyte variations on resting hospital patients and obtained results quite similar to those published by Shaw (1927).

These authors obtained evidence of diurnal variations in the leucocyte count. When their values are plotted the resulting curves resemble the curves showing lactic acid changes. In all studies there is a mid-day rise in leucocytes with considerable variation from hour to hour. In fact, Sabin et al. (1925) speak of an

"hourly rhythm of leucocytes." In extended studies there is also a mid-night swell. The late afternoon rise is present in Shaw's series but comes an hour later in Tschishkow's. Figure 8 gives a comparison of both the above series.

In Table XVII the counts made by Shaw on man are listed for each hour and their mean values recorded. Figure 5 shows the diurnal variations in leucocytes along with the lactic acid mean hourly values for Group A (leucocytes in man and lactic acid in dogs).

These curves are strikingly similar in their time relationships. One curve coincides quite well with the other provided that the lactic acid values be moved one hour later. An exception should be made in the case of the 1 A.M. peak which is identical for both variables. The smoothed curves for both variables show the similarity to an even more pronounced degree.

This marked likeness may be merely an accidental resemblance. Without direct evidence concerning the relationship of the two variables, one would be unjustified in assigning to one the role of cause and to the other, effect; or to both the result of a common cause.

An attractive hypothesis would be to consider the lactic acid in the blood of the resting organism, in part, the result of the glycolytic activity of the white cells. It is well known that one of the potentialities of the white cell, particularly the neutrophil, is the

production of lactic acid from carbohydrate.

Levene and Meyer (1912) first demonstrated leucocytic splitting of dextrose in vitro. Furthermore, it has been demonstrated by Glover, Daland and Schmitz (1930) that the metabolism of normal white blood cells resembles that of malignant tissue as indicated by Warburg (1926) who attributed small $O_2$ consumption but large aerobic and anerobic glycolytic powers to malignancies. Maclean and Weir (1915) demonstrated that both erythrocytes and leucocytes can glycolyze dextrose in vitro but that the ratio of the activity of leucocytes to erythrocytes, cell for cell, varies roughly from 200:1 up to 1000:1 in favor of the leucocytes. Maclean and Weir conclude that, in normal blood, the white cells probably exert greater glycolytic effect than the red cells even though they are present in much smaller numbers. Falcon-Lesses (1927) proved that leukemic blood in vitro, displays greater glycolytic activity than normal blood.

Considering, then, the above demonstrated facts regarding leucocytes and other variations, one may be justified in adopting as a provisional hypothesis the conception that, lactic acid changes in the blood of the resting organism are, in a measure, dependent upon the variations in numbers of leucocytes. This hypothesis must be regarded as provisional until further work of a direct character determines whether or not it can be regarded as valid.

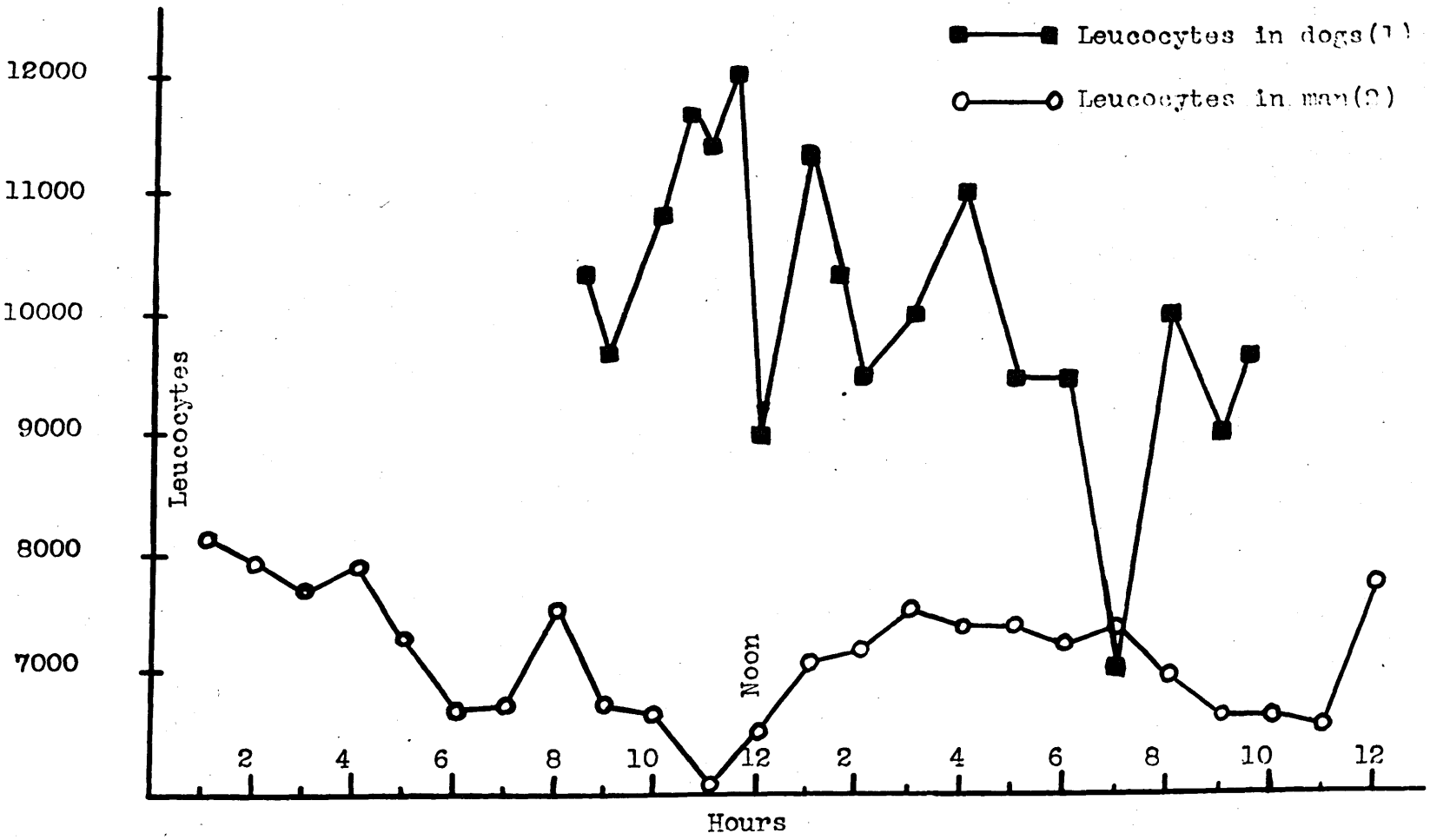


Figure 8. Diurnal variation in leucocytes. Data from (1) Tschistikow, W.G., Folia Haematologica, 1927, 34, 125; (2) Shaw, A.F.B., Journal Path. and Bact., 1927, 30, 1.

## SUMMARY

1. There is a well-defined diurnal variation in the blood lactic acid of the resting, normal dog.

2. The twenty-four hour period exhibits two crests. One at mid-day lasts well into the afternoon. The other occurs approximately at midnight and is of shorter duration than the one at mid-day. It may be, however, of greater magnitude than the one at noon.

3. A third crest is observed in late afternoon near 6 to 7 P.M. This peak is usually shorter, more spiked in appearance and more inconstant in occurrence.

4. Data from other authors are presented showing a similarity between the changes in concentration of leucocytes and lactic acid during the twenty-four hour period.

5. A provisional hypothesis linking the two phenomena is suggested.

TABLE IV

Number, weight, sex and condition of dogs used in study of diurnal variation of blood lactic acid

| No. Of Dog | Name of Dog | Sex | Wt. in Kgms. | Condition |
|---|---|---|---|---|
| | | Group A | | |
| 1 | Setter | Female | 13.0 | Very thin |
| 2 | Jean | Female | 10.2 | Thin |
| 3 | Titan | Female | 12.5 | Very thin |
| 4 | Beth | Female | 13.4 | Good |
| 5 | Keche | Female | 13.7 | Good |
| 6 | Blackie | Male | 9.0 | Good |
| 7 | Biggie | Male | 24.0 | Good |
| 8 | Shep | Male | 23.6 | Thin |
| 9 | Champ | Male | 23.0 | Thin |
| 10 | Shag | Male | 11.8 | Good |
| | | Groups B and C | | |
| 11 | Dacksie# | Female | 10.2 | Good |
| 12 | Brindle | Female | 9.8 | Good |
| 13 | Spot | Female | 9.8 | Good |
| 14 | Blimp* | Male | 7.6 | Very thin |
| 15 | Jack | Male | 10.7 | Good |
| 16 | Brownie | Male | 9.0 | Good |
| 17 | Bob | Male | 9.1 | Good |

*Died 2/16/40
#Whelped 8/20/40

TABLE V

Hourly values for blood lactic acid
in mgms. per cent

GROUP A

| | Dog #1 | | Dog #6 | | Dog #7 | |
|---|---|---|---|---|---|---|
| | Feb. 9, 1940 | | March 7, 1940 | | April 12, 1940 | |
| Time | A.M. | P.M | A.M. | P.M. | A.M. | P.M. |
| 1 | 17.22 | 11.80 | - - | 15.88 | 7.12 | 2.45 |
| 2 | 14.85 | 10.49 | 6.23 | 1.98 | 6.73 | 9.82 |
| 3 | 13.06 | 12.90 | 5.34 | 10.75 | 6.73 | 5.74 |
| 4 | 16.43 | 12.77 | 6.93 | 14.75 | 5.34 | 4.15 |
| 5 | 11.62 | 13.76 | 9.70 | 10.30 | 7.32 | 5.74 |
| 6 | 9.30 | 13.54 | 7.80 | 26.13 | 8.71 | 6.57 |
| 7 | 14.15 | 15.64 | 4.65 | 10.10 | 8.51 | 4.51 |
| 8 | 11.04 | 13.26 | -- | 8.11 | 8.71 | 6.59 |
| 9 | 12.21 | 15.64 | 5.30 | 7.62 | 6.93 | -- |
| 10 | 10.09 | 17.93 | 3.60 | 8.61 | 6.73 | 10.85 |
| 11 | 6.55 | 15.54 | 17.22 | 6.53 | 5.70 | 10.09 |
| 12 | 8.11 | 10.69 | 15.04 | 7.72 | 10.89 | 11.28 |
| Total: | | 308.59 | | 210.29 | | 167.21 |
| Average: | | 12.86 | | 9.56 | | 7.27 |
| Range: | | 6.55 to 17.22 | | 1.98 to 26.13 | | 5.34 to 11.28 |

TABLE VI

Hourly values for blood lactic acid
in mgms. per cent

GROUP A, cont'd.

| | Dog #7 | | Dog #2 | | Dog #7 | |
|---|---|---|---|---|---|---|
| | May 10, 1940 | | June 14, 1940 | | July 10, 1940 | |
| | A.M. | P.M. | A.M. | P.M | A.M. | P.M. |
| 1 | 3.52 | 5.48 | 17.28 | 10.78 | 7.03 | 8.51 |
| 2 | -- | 6.93 | 10.09 | 11.68 | 3.30 | 14.85 |
| 3 | 5.74 | 7.82 | 12.47 | 7.32 | 8.90 | 12.07 |
| 4 | 7.92 | 5.34 | 9.30 | 8.11 | 11.10 | 5.74 |
| 5 | 2.91 | 5.34 | 8.31 | -- | 2.80 | 10.70 |
| 6 | 5.99 | 4.95 | 18.81 | 18.26 | 4.80 | -- |
| 7 | 2.37 | 5.74 | 11.68 | -- | 7.80 | 7.30 |
| 8 | -- | 4.15 | 14.05 | 9.72 | 2.90 | 3.36 |
| 9 | 3.56 | 6.33 | 9.10 | 8.50 | 6.90 | 5.34 |
| 10 | 5.94 | 4.95 | 6.73 | 8.31 | 6.70 | 6.14 |
| 11 | 4.95 | 5.34 | 5.44 | 24.78 | 7.92 | 9.70 |
| 12 | 5.90 | 4.35 | 10.39 | 9.90 | 6.90 | 10.10 |
| Total: | 115.52 | | 251.01 | | 170.86 | |
| Average: | 5.25 | | 11.41 | | 7.43 | |
| Range: | 2.37 to 7.92 | | 5.44 to 24.78 | | 2.80 to 14.85 | |

TABLE VII

Hourly values for blood lactic acid
in mgms. per cent

GROUP A, cont'd.

| | Dog #3 | | Dog #8 | | Dog #4 | |
|---|---|---|---|---|---|---|
| | August 7,1940 | | Sept. 11,1940 | | Oct. 12,1940 | |
| | A.M. | P.M. | A.M. | P.M. | A.M. | P.M. |
| 1 | 9.70 | 8.31 | 8.71 | 6.14 | 21.98 | 16.24 |
| 2 | 10.03 | 7.52 | 3.47 | 4.36 | 18.81 | 8.71 |
| 3 | 12.07 | 4.91 | 3.17 | 6.34 | 19.21 | 14.85 |
| 4 | 9.90 | 4.16 | 3.56 | 5.54 | 16.83 | 8.32 |
| 5 | 7.72 | 3.96 | 3.96 | 3.76 | 18.41 | 17.23 |
| 6 | 9.30 | 9.11 | 3.17 | 2.77 | 16.83 | 9.50 |
| 7 | 8.91 | 8.91 | 2.77 | 1.98 | 17.82 | 13.66 |
| 8 | 7.72 | 12.07 | 3.47 | 2.38 | 13.27 | 14.06 |
| 9 | 11.68 | 8.71 | 6.53 | 2.57 | 12.67 | 17.62 |
| 10 | 10.03 | 9.50 | 7.72 | 3.47 | 10.50 | 24.55 |
| 11 | 11.88 | 15.04 | 3.37 | 2.38 | 20.39 | 20.99 |
| 12 | 7.92 | 13.46 | 4.55 | 3.47 | 18.27 | 23.76 |
| Total: | | 222.52 | | 99.61 | | 394.48 |
| Average: | | 9.28 | | 4.15 | | 16.44 |
| Range: | | 3.96 to 15.04 | | 1.98 to 8.71 | | 8.32 to 24.55 |

TABLE VIII

Hourly values for blood lactic acid in mgms. per cent

GROUP A, cont'd.

| | Dog #9 | | Dog #5 | | Dog #10 | |
|---|---|---|---|---|---|---|
| | Nov. 9,1940 | | Dec. 7,1940 | | Jan. 3,1941 | |
| | A.M. | P.M. | A.M. | P.M. | A.M. | P.M. |
| 1 | 21.48 | 17.22 | 6.53 | 28.11 | 7.70 | 5.10 |
| 2 | 16.73 | 18.02 | 8.31 | 19.99 | 5.30 | 6.90 |
| 3 | 17.22 | 16.73 | 6.73 | 20.59 | 7.50 | 7.30 |
| 4 | 16.63 | 22.97 | 7.52 | 17.03 | 6.10 | 6.50 |
| 5 | 12.27 | 21.78 | 10.39 | 9.12 | 9.70 | 3.80 |
| 6 | 11.28 | 20.19 | 8.31 | 9.31 | 6.10 | 4.10 |
| 7 | 11.28 | 15.64 | 9.60 | 10.19 | 3.60 | 8.30 |
| 8 | 11.28 | 20.00 | 8.12 | 6.73 | 5.50 | 6.70 |
| 9 | 13.56 | 17.62 | -- | 6.73 | 8.30 | 8.90 |
| 10 | 17.32 | 20.79 | 31.28 | 3.76 | 6.90 | 8.10 |
| 11 | 16.43 | 16.23 | 28.11 | 5.74 | 9.50 | 9.30 |
| 12 | 17.82 | 15.64 | 24.94 | 3.76 | 8.30 | 7.50 |
| Total: | 406.13 | | 290.90 | | 167.00 | |
| Average: | 16.92 | | 12.65 | | 6.96 | |
| Range: | 11.28 to 22.97 | | 3.76 to 31.28 | | 3.60 to 9.70 | |

TABLE IX

Dog #11 *

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Low | High | Total | No. of Samples |
|---|---|---|---|---|---|---|---|---|
| Dec. '39 | 9.9 (7pm) | -- | 17.66 (7am) | 13.46 (1pm) | 9.9 | 17.66 | 41.02 | 3 |
| Jan. '41 | -- | 9.50 (8pm) | 8.30 (2am) | 12.30 (8am) | 8.30 | 12.30 | 30.10 | 3 |
| Feb. '40 | 23.56 (1pm) | 27.32 (7pm) | 38.80 (1am) | 16.47 (7am) | 16.47 | 38.80 | 106.15 | 4 |
| Mar. '40 | 8.7 (1pm) | 12.4 (7pm) | 19.8 (1am) | 10.69 (7am) | 8.7 | 19.80 | 51.59 | 4 |
| Apr. '40 | 9.72 (1pm) | 7.90 (7pm) | -- | 9.60 (7am) | 7.90 | 9.60 | 27.22 | 3 |
| May. '40 | 8.80 (1pm) | 5.54 (7pm) | 5.74 (1am) | 6.67 (7am) | 5.54 | 8.80 | 26.75 | 4 |
| June '40 | 10.09 (7am) | 6.32 (1pm) | 16.83 (7pm) | 3.76 (1am) | 3.76 | 16.83 | 37.00 | 4 |
| July '40 | 11.38 (1pm) | 6.53 (7pm) | 7.13 (1am) | 5.34 (7am) | 5.34 | 11.38 | 30.38 | 4 |
| Aug. '40 | 19.20 (1pm) | 14.25 (7pm) | 11.08 (1am) | 8.31 (7am) | 8.31 | 19.20 | 52.84 | 4 |
| Sept. '40 | 16.63 (12m) | 11.28 (6pm) | 11.88 (12) | 15.64 (6am) | 11.28 | 16.63 | 55.43 | 4 |
| Oct. '40 | 10.69 (5pm) | 16.43 (11p) | 7.52 (5am) | 8.71 (11a) | 7.52 | 16.43 | 43.35 | 4 |
| Nov. '40 | 3.17 (4pm) | 9.30 (10p) | 14.45 (4am) | 11.10 (10a) | 3.17 | 14.45 | 38.02 | 4 |
| Dec. '40 | 11.48 (3pm) | 9.90 (9pm) | 9.70 (3am) | 10.69 (9am) | 9.70 | 11.48 | 41.77 | 4 |
| | Range: 3.17 -- 38.80 | | | | Total | | 581.62 | 49 |
| | | | | | Average | | 11.87 | |

*Dog whelped 8/20/40

TABLE X

Dog #12

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Low | High | Total | No. of Samples |
|---|---|---|---|---|---|---|---|---|
| Dec. '39 | 10.40 (5pm) | 12.31 (11pm | 10.37 (5am) | 14.55 (11am | 10.37 | 14.55 | 47.63 | 4 |
| Jan. '41 | 6.7 (12m) | 7.9 (6pm) | 8.3 (12) | 7.9 (6am) | 6.7 | 8.3 | 30.80 | 4 |
| Feb. '40 | 18.70 (5pm) | 12.67 (11pm | 17.7 (5am) | 14.45 (11am | 12.67 | 18.70 | 63.52 | 4 |
| Mar. '40 | 4.50 (11am | 10.8 (5pm) | -- | 12.17 (5am) | 4.50 | 12.17 | 27.47 | 3 |
| Apr. '40 | 8.17 (5pm) | 9.06 (11pm | 5.74 (5am) | 8.90 (11am | 5.74 | 9.06 | 31.87 | 4 |
| May '40 | 10.33 (tpm) | 7.32 (11pm | 4.95 (5am) | 6.33 (11am | 4.95 | 10.33 | 28.93 | 4 |
| June '40 | 11.28 (11am | 6.73 (5pm) | 4.55 (11pm | 3.16 (5am) | 4.55 | 11.28 | 25.72 | 4 |
| July '40 | 9.10 (5pm) | 6.63 (11pm | 8.71 (5am) | 6.83 (11am | 6.63 | 9.10 | 31.27 | 4 |
| Aug. '4C | 12.27 (11am | 15.24 (5pm) | 7.92 (11pm | 6.93 (5am) | 6.93 | 15.24 | 42.36 | 4 |
| Sept. '40 | 9.10 (10am | 7.92 (4pm) | 3.76 (10pm | 5.54 (4am) | 3.76 | 9.10 | 26.32 | 4 |
| Oct. '40 | 5.34 (2pm) | 11.09 (9pm) | 11.88 (3am) | 8.51 (9am) | 5.34 | 11.88 | 36.82 | 4 |
| Nov. '40 | 11.48 (2pm) | 21.58 (8pm) | -- | 8.71 (8am) | 8.71 | 21.58 | 41.77 | 3 |
| Dec. '40 | 13.07 (1pm) | 17.62 (7pm) | 12.27 (1am) | 12.17 (7am) | 12.17 | 17.62 | 55.13 | 4 |
| | Range: | 3.76 -- 21.58 | | | Total | | 489.61 | 50 |
| | | | | | Average | | 9.8 | |

TABLE XI
Dog #13

| | Sample 1 | 2 | 3 | 4 | Low | High | Total | No. of Samples |
|---|---|---|---|---|---|---|---|---|
| Dec. '39 | 34.45 (4pm) | -- | 11.89 (4am) | 15.99 (10am | 11.89 | 34.45 | 62.33 | 3 |
| Jan. '41 | 4.10 (11am | 12.70 (5pm) | 9.50 (11pm | 8.10 (5am) | 4.10 | 12.70 | 34.40 | 4 |
| Feb. '40 | 27.12 (4pm) | 17.02 (10pm | 15.80 (4am) | 19.80 (10am | 15.80 | 27.12 | 79.74 | 4 |
| Mar. '40 | 11.70 (4pm) | 10.98 (10pm | 9.99 (4am) | 7.56 (10am | 7.56 | 11.70 | 40.23 | 4 |
| Apr. '40 | 11.78 (4pm) | 6.57 (10pm | 5.74 (4am) | 7.62 (10am | 5.74 | 11.78 | 31.71 | 4 |
| May '40 | 11.44 (4pm) | 7.72 (10pm | 3.16 (4am) | 8.71 (10am | 3.16 | 11.44 | 31.03 | 4 |
| June '40 | 7.52 (10am | 9.70 (4pm) | -- | 4.16 (4am) | 4.16 | 9.70 | 21.38 | 3 |
| July '40 | 14.25 (4pm) | 9.70 (10pm | 8.40 (4am) | -- | 8.40 | 14.25 | 32.35 | 3 |
| Aug. '40 | 12.47 (10am | 12.07 (4pm) | 8.31 (10pm | 8.31 (4am) | 8.31 | 12.47 | 41.16 | 4 |
| Sept. '40 | 9.90 (9am) | 9.50 (3pm) | 5.34 (9pm) | 3.36 (3am) | 3.36 | 9.90 | 28.10 | 4 |
| Oct. '40 | 4.95 (2pm) | 12.76 (8pm) | 10.89 (2am) | 8.9 (8am) | 4.95 | 12.76 | 37.50 | 4 |
| Nov. '40 | 8.12 (1pm) | 24.15 (7pm) | 13.56 (1am) | 11.58 (7am) | 8.12 | 24.15 | 57.41 | 4 |
| Dec. '40 | 8.71 (12m) | 16.63 (6pm) | 11.28 (12) | 10.00 (6am) | 8.71 | 16.63 | 46.62 | 4 |
| | Range: 3.16 -- 34.45 | | | | Total | | 543.96 | 49 |
| | | | | | Average | | 11.1 | |

## TABLE XII

Dogs #14* and 15

Dog #14 was used during December, 1939 and February, 1940. All other samples were from dog #15.

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Low | High | Total | No. of Samples |
|---|---|---|---|---|---|---|---|---|
| Dec. '39 | 11.88 (8pm) | -- | 28.01 (8am) | 14.85 (2pm) | 11.88 | 28.01 | 54.74 | 3 |
| Jan. '41 | 3.2 (3pm) | 5.1 (9pm) | 7.5 (3am) | 10.9 (9am) | 3.2 | 10.9 | 26.70 | 4 |
| Feb. '40 | 17.56 (2pm) | 23.95 (8pm) | 17.6 (2am) | 18.09 (8am) | 17.56 | 23.95 | 77.20 | 4 |
| Mar. '40 | 7.7 (2pm) | 12.7 (8pm) | 8.11 (2am) | -- | 7.70 | 12.70 | 28.51 | 3 |
| Apr. '40 | 13.76 (2pm) | 13.60 (8pm) | 8.91 (2am) | 6.63 (8am) | 6.63 | 13.76 | 42.90 | 4 |
| May '40 | 12.53 (2pm) | 3.17 (8pm) | 5.91 (2am) | 7.48 (8am) | 3.17 | 12.53 | 29.09 | 4 |
| June '40 | 5.74 (8am) | 9.10 (2pm) | 9.50 (8pm) | 4.35 (2am) | 4.35 | 9.50 | 28.69 | 4 |
| July '40 | 16.53 (2pm) | 8.32 (8pm) | 10.90 (2am) | 10.30 (8am) | 8.32 | 16.53 | 46.05 | 4 |
| Aug. '40 | 22.96 (2pm) | 6.[illegible] (8pm) | [illegible] (2am) | 4.75 (8am) | 4.75 | 22.96 | 40.77 | 4 |
| Sept. '40 | 6.93 (1pm) | -- | 5.34 (1am) | 4.35 (7am) | 4.35 | 6.93 | 16.62 | 3 |
| Oct. '40 | 6.73 (12m) | 6.73 (6pm) | 5.94 (12) | 7.13 (6am) | 5.94 | 7.13 | 26.53 | 4 |
| Nov. '40 | 13.46 (11am | 7.92 (5pm) | 10.10 (11pm | 9.50 (5am) | 7.92 | 13.46 | 40.98 | 4 |
| Dec. '40 | 7.72 (4pm) | 6.63 (10pm | 9.70 (4am) | 9.11 (10am | 6.63 | 9.70 | 33.16 | 4 |

Total:

Range: #14, 11.88 - 28.01 — #14, 131.94 — 7

#15, 3.2 - 22.96 — #15, 360.0 — 42

Average:

#14, 18.85

#15 8.57

* Dog died 2/16/40

TABLE XIII

Dog #16

| | Sample 1 | 2 | 3 | 4 | Low | High | Total | No. of Samples |
|---|---|---|---|---|---|---|---|---|
| Dec. '39 | 15.84 (3pm) | -- | 6.73 (3am) | 9.40 (9am) | 6.73 | 15.84 | 31.97 | 3 |
| Jan. '41 | 6.5 (4pm) | 8.9 (10pm | 9.1 (4pm) | 12.1 (10am | 6.50 | 12.1 | 27.50 | 4 |
| Feb. '40 | 20.19 (3pm) | 2.43 (9pm) | 11.48 (3am) | 11.24 (9am) | 2.43 | 20.19 | 45.34 | 4 |
| Mar. '40 | 16.03 (3pm) | 12.96 (9pm) | 5.84 (3am) | 14.55 (9am) | 5.84 | 16.03 | 49.38 | 4 |
| Apr. '40 | 8.91 (3pm) | 8.91 (9pm) | 8.91 (3am) | 9.50 (9am) | 8.91 | 9.50 | 36.23 | 4 |
| May '40 | 8.11 (3pm) | 5.54 (9pm) | 10.23 (3am) | 8.71 (9am) | 5.54 | 10.23 | 32.59 | 4 |
| June '40 | 8.51 (3pm) | 5.94 (9pm) | 5.74 (3am) | 3.96 (9am) | 3.96 | 8.51 | 24.15 | 4 |
| July '40 | 11.08 (3pm) | 3.76 (9pm) | 12.37 (3am) | 5.3 (9am) | 3.76 | 12.37 | 32.51 | 4 |
| Aug. '40 | 11.48 (9am) | 6.73 (3pm) | 6.73 (9pm) | 10.29 (3am) | 6.73 | 11.48 | 35.23 | 4 |
| Sept. '40 | 4.35 (2pm) | 7.52 (8pm) | 4.15 (2am) | 4.75 (8am) | 4.15 | 7.52 | 20.77 | 4 |
| Oct. '40 | 10.69 (1pm) | 12.47 (7pm) | 12.47 (1am) | 17.62 (7am) | 10.69 | 17.62 | 53.25 | 4 |
| Nov. '40 | 15.24 (12m) | 15.94 (6pm) | 18.61 (12) | 13.27 (9am) | 13.27 | 18.61 | 63.06 | 4 |
| Dec. '40 | -- | 9.30 (5pm) | 12.97 (11pm | 6.53 (5am) | 6.53 | 12.97 | 28.80 | 3 |
| | Range: 2.43 - 18.61 | | | | Total: | | 480.70 | 50 |
| | | | | | Average: | | 9.71 | |

TABLE XIV

Dog #17

| | Sample 1 | 2 | 3 | 4 | Low | High | Total | No. of Samples |
|---|---|---|---|---|---|---|---|---|
| Dec. '39 | 4.25 (6pm) | 11.01 (12) | 10.99 (6am) | 7.82 (12m) | 4.25 | 11.01 | 34.07 | 4 |
| Jan. '41 | 12.30 (1pm) | 12.10 (7pm) | 4.30 (1am) | 6.90 (7am) | 4.30 | 12.30 | 35.60 | 4 |
| Feb. '40 | 20.98 (6pm) | 11.24 (12) | 14.25 (6am) | 14.65 (12m) | 11.24 | 20.98 | 61.12 | 4 |
| Mar. '40 | 9.02 (12m) | 10.50 (6pm) | 10.69 (12) | 7.72 (6am) | 7.72 | 10.69 | 38.13 | 4 |
| Apr. '40 | 8.47 (6pm) | 9.58 (12) | 5.14 (6am) | 11.08 (12m) | 5.14 | 11.08 | 34.27 | 4 |
| May '40 | 7.48 (6pm) | 10.29 (12) | 3.16 (6am) | 12.67 (12m) | 3.16 | 12.67 | 33.60 | 4 |
| June '40 | -- | 7.92 (6pm) | 6.10 (12) | 8.31 (6am) | 6.10 | 8.31 | 22.33 | 3 |
| July '40 | -- | 7.54 (12) | 8.71 (6am) | 9.20 (12m) | 7.54 | 9.20 | 25.45 | 3 |
| Aug. '40 | 6.93 (12m) | 6.33 (6pm) | 9.10 (12) | 8.51 (6am) | 6.33 | 9.10 | 30.87 | 4 |
| Sept. '40 | 8.12 (11am) | 4.55 (5pm) | 8.54 (11pm | 4.75 (5am) | 4.55 | 8.54 | 25.96 | 4 |
| Oct. '40 | 9.10 (4pm) | 2.77 (10pm | 9.10 (4am) | 8.71 (10am | 2.77 | 9.10 | 29.68 | 4 |
| Nov. '40 | 17.22 (3pm) | 10.10 (9pm) | 14.65 (3am) | 9.50 (9am) | 9.50 | 17.22 | 51.47 | 4 |
| Dec. '40 | 13.36 (2pm) | 20.39 (8pm) | 11.58 (2am) | 10.09 (8am) | 10.09 | 20.39 | 55.42 | 4 |
| | Range: 2.77 - 20.98 | | | | Total: | | 477.97 | 50 |
| | | | | | Average: | | 9.56 | |

TABLE XV
SUMMARY OF INDIVIDUAL AND GROUP PERFORMANCE.

| No. of Dog | Weight (Kg) | No. of Samples | Total Lactic Acid in Mgrms % | Average | No. of Dog | Weight (Kg) | No. of Samples | Total Lactic Acid in Mgrms % | Average |
|---|---|---|---|---|---|---|---|---|---|
| | | | Group A | | | | | | |
| | Females | | | | | Males | | | |
| 1 | 13.0 | 24 | 308.59 | 12.86 | 6 | 9.0 | 22 | 210.29 | 9.56 |
| 2 | 13.7 | 22 | 251.01 | 11.41 | 7 | 24.0 | 23 | 167.21 | 7.27 |
| 3 | 12.5 | 24 | 222.52 | 9.28 | 7 | 24.0 | 22 | 115.52 | 5.25 |
| 4 | 13.4 | 24 | 394.48 | 16.44 | 7 | 24.0 | 23 | 170.86 | 7.43 |
| 5 | 13.7 | 23 | 290.90 | 12.65 | 8 | 23.6 | 24 | 99.61 | 4.15 |
| | | | | | 9 | 23.0 | 24 | 406.13 | 16.92 |
| | | | | | 10 | 11.8 | 24 | 167.00 | 6.96 |
| Total | | 117 | 1,467.50 | 12.53 | | | 162 | 1,336.62 | 8.25 |
| | | | Groups B and C | | | | | | |
| | Females | | | | | Males | | | |
| 11 | 10.2 | 49 | 543.96 | 11.10 | 14 | 7.6 | 7 | 131.94 | 18.85 |
| 12 | 9.8 | 50 | 489.61 | 9.80 | 15 | 10.7 | 42 | 360.00 | 8.57 |
| 13 | 9.8 | 49 | 543.96 | 11.10 | 16 | 9.0 | 50 | 480.70 | 9.71 |
| | | | | | 17 | 9.1 | 50 | 477.97 | 9.56 |
| Total | | 148 | 1,615.19 | 10.91 | | | 149 | 1,450.61 | 9.74 |

Ave. for all Females 11.63

Ave. for all Males 8.96

Ave. for Group A 10.05

Ave. for Group B&C 10.32

TABLE XVI

Average hour values from dogs in Group A

| Hour | A.M. | P.M. |
|---|---|---|
| ½ | 11.67 | 11.33 |
| 2 | 9.44 | 10.84 |
| 3 | 9.85 | 10.61 |
| 4 | 9.80 | 9.61 |
| 5 | 8.76 | 9.60 |
| 6 | 9.20 | 11.31 |
| 7 | 8.60 | 9.27 |
| 8 | 8.60 | 8.93 |
| 9 | 8.79 | 9.60 |
| 10 | 10.30 | 10.58 |
| 11 | 11.45 | 11.79 |
| 12 | 11.59 | 10.13 |

Average hour values from dogs Group B (Dec. - July).

| Hour | A.M. | P.M. |
|---|---|---|
| 1 | 15.04 | 11.74 |
| 2 | 9.16 | 13.15 |
| 3 | 8.50 | 12.30 |
| 4 | 8.45 | 17.21 |
| 5 | 8.97 | 9.81 |
| 6 | 8.31 | 8.60 |
| 7 | 10.93 | 12.35 |
| 8 | 12.71 | 11.89 |
| 9 | 9.60 | 6.56 |
| 10 | 11.20 | 10.40 |
| 11 | 9.55 | 8.76 |
| 12 | 10.74 | 9.50 |

Average hour values from dogs in Group C (Aug. - Jan.)

| Hour | A.M. | P.M. |
|---|---|---|
| 1 | 9.84 | 11.72 |
| 2 | 8.29 | 11.42 |
| 3 | 9.57 | 8.91 |
| 4 | 9.37 | 7.75 |
| 5 | 7.22 | 10.23 |
| 6 | 10.41 | 10.80 |
| 7 | 10.15 | 16.12 |
| 8 | 8.25 | 13.05 |
| 9 | 10.16 | 8.04 |
| 10 | 10.43 | 6.61 |
| 11 | 9.33 | 10.91 |
| 12 | 10.16 | 10.85 |

TABLE XVII

Diurnal variation of leucocytes in man*

| | Case 9 | Case 10 | Case 11 | Case 12 | Ave. |
|---|---|---|---|---|---|
| A.M. 1 | 8,200 | 7,580 | 7,350 | 9,000 | 8,100 |
| 2 | 8,200 | 8,350 | 6,700 | 8,350 | 7,900 |
| 3 | 8,350 | 7,600 | 6,700 | 7,850 | 7,600 |
| 4 | 8,200 | 8,600 | 6,750 | 7,850 | 7,850 |
| 5 | 7,000 | 6,600 | 6,850 | 8,350 | 7,200 |
| 6 | 6,700 | 7,000 | 5,800 | 6,950 | 6,600 |
| 7 | 7,000 | 6,700 | 6,050 | 7,300 | 6,760 |
| 8 | 7,550 | 8,300 | 6,500 | 8,000 | 7,580 |
| 9 | 6,400 | 6,700 | 6,500 | 7,200 | 6,700 |
| 10 | 6,000 | 5,800 | 6,300 | 8,400 | 6,600 |
| 11 | 6,050 | 6,400 | 5,200 | 6,750 | 6,100 |
| 12 | 6,500 | 6,750 | 5,950 | 6,800 | 6,500 |
| P.M. 1 | 6,400 | 8,200 | 6,600 | 7,300 | 7,100 |
| 2 | 6,900 | 7,800 | 6,150 | 8,000 | 7,200 |
| 3 | 7,300 | -- | 6,950 | 8,300 | 7,500 |
| 4 | 7,200 | -- | 6,900 | 8,200 | 7,400 |
| 5 | 7,200 | 7,800 | 7,500 | 7,250 | 7,400 |
| 6 | 6,750 | 8,050 | 7,500 | 6,650 | 7,240 |
| 7 | 7,900 | 7,500 | 7,850 | 6,600 | 7,400 |
| 8 | 6,750 | 6,850 | 6,900 | 7,000 | 6,900 |
| 9 | 6,400 | 7,250 | 6,200 | 6,700 | 6,600 |
| 10 | 6,600 | 7,000 | 6,300 | 6,700 | 6,650 |
| 11 | 7,700 | 6,200 | 5,600 | 6,400 | 6,500 |
| 12 | 7,700 | 7,000 | 8,000 | 8,000 | 7,700 |

*Shaw, A.F.B., Jour. Path. & Bact., 1927, 30,1-19.

# SEASONAL TEMPERATURE AND BLOOD LACTIC ACID

# INTRODUCTION

Some data have been reported in the literature which bears more or less directly on the problem of the effect of temperature variation and the blood lactic acid concentration. Yamaga (1937) subjected mature, healthy rabbits to carefully controlled temperatures for varying periods of time. When the temperature was 40C or 50C, he obtained considerable increase in blood lactic acid. Although the animals remained in the high temperatures from thirty minutes to one hour, it required some three to six hours for the lactic acid to return to the resting level.

Truka-Tuzon (1940) made ten to twenty determinations of blood lactic acid per month on different persons throughout a nine-month period from September through June. His lactic acid values, averaged by months, correlated closely with the mean monthly temperature, in that a reduction or increase in temperature, as expressed by the monthly mean, was accompanied by a decrease or increase, respectively, in lactic acid.

Dill et al. (1940) found an increase in concentration of blood lactic acid of approximately fifty per cent when determined in Benoit, Mississippi as compared to the values found in the same human subjects in Boston, Massachusetts.

In view of these findings, it was thought that investigation of the lactic acid concentration in the blood of healthy dogs might prove of some interest. In addition,

it was believed, subjects which were used continuously throughout the entire experimental period would yield more valuable information than different subjects chosen for each sampling.

## PROCEDURE

Two dogs, a male, number 25, weighing 24 Kgm. and a female, number 30, weighing 10.2 Kgm., were confined in a small, unheated, but fully enclosed house constructed of light wooden sheeting. This structure offered complete shelter from wind, rain and snow but afforded little protection to temperature changes. The inside temperature followed within 1 to 3 degrees centigrade the outside temperature.

The thermometer used was the Bristol Recording Thermometer. Continuous temperatures throughout the twenty-four hour period were thus available. This instrument provides readings which serve as the semi-official temperatures for the community.

Uniform feeding and watering procedures were used for the dogs. The food was a balanced, commercial product which was given to them once each day at 5 P.M. The dogs' condition improved during the experiment, indicating a healthy state at all times.

Samples were taken from the peripheral leg veins, all four legs being used in rotation. The method used in obtaining the blood and determining the lactate content is

described in the section on the effect of hemorrhage on blood lactic acid. All samples were taken at 11 A.M. every day of the week except Sunday and holidays. Approximately 12 c.c. of blood were removed each time a sample was obtained. Each dog was bled every other day. That such a blood loss was in no way injurious was indicated by the improvement of each dogs' condition and vigor.

All samples were run in duplicate, the results agreeing within two mgm. per cent in practically all cases. All blood filtrates were analyzed within a week after removal from the dog, and in the interim were kept in stoppered tubes in the refrigerator.

To insure a state of complete rest in the experimental animal, both dogs were leashed for at least forty-five minutes previous to the time of blood withdrawal. No form of activity was permitted during this rest period.

The experiment was started early in the Fall when the temperature daily reached a peak almost as high as during the hottest part of Summer. Samples were taken every other day from each dog for a period of six months. During this interval, temperatures approximating those of hottest Summer as well as those of coldest Winter were registered.

## RESULTS

In Table XVIII a complete record of the data collected in the experiment is given by months for each of the two dogs for the entire six months period. Only the 11 A.M. temperatures of the days on which samples were taken are given, for it was at this hour that all samples were drawn. Duplicate determinations were run on practically all samples. Each lactic acid value listed in the table respresents an average of the two analyses, except for a few in which the duplicates were lost. In those cases the single values were taken, and are so indicated in Table XVIII.

Figure 9 represents, graphically, the lactic acid and temperature values contained in Table XVIII. On the days when no samples were taken, a notation is entered. The graph includes all values taken during the six months' period.

The first impression is that dog number 30 exhibits a much greater range of variation than does dog number 25. It must be remembered that between two given points on a lactic acid curve there may be an interval of two days instead of one. This is due to the fact that no samples were taken on Sunday, hence in the case of dog number 30 a sample was taken on Saturday, September 14, and no further sample obtained until Tuesday, September 17. The missed day is not included on the time scale,

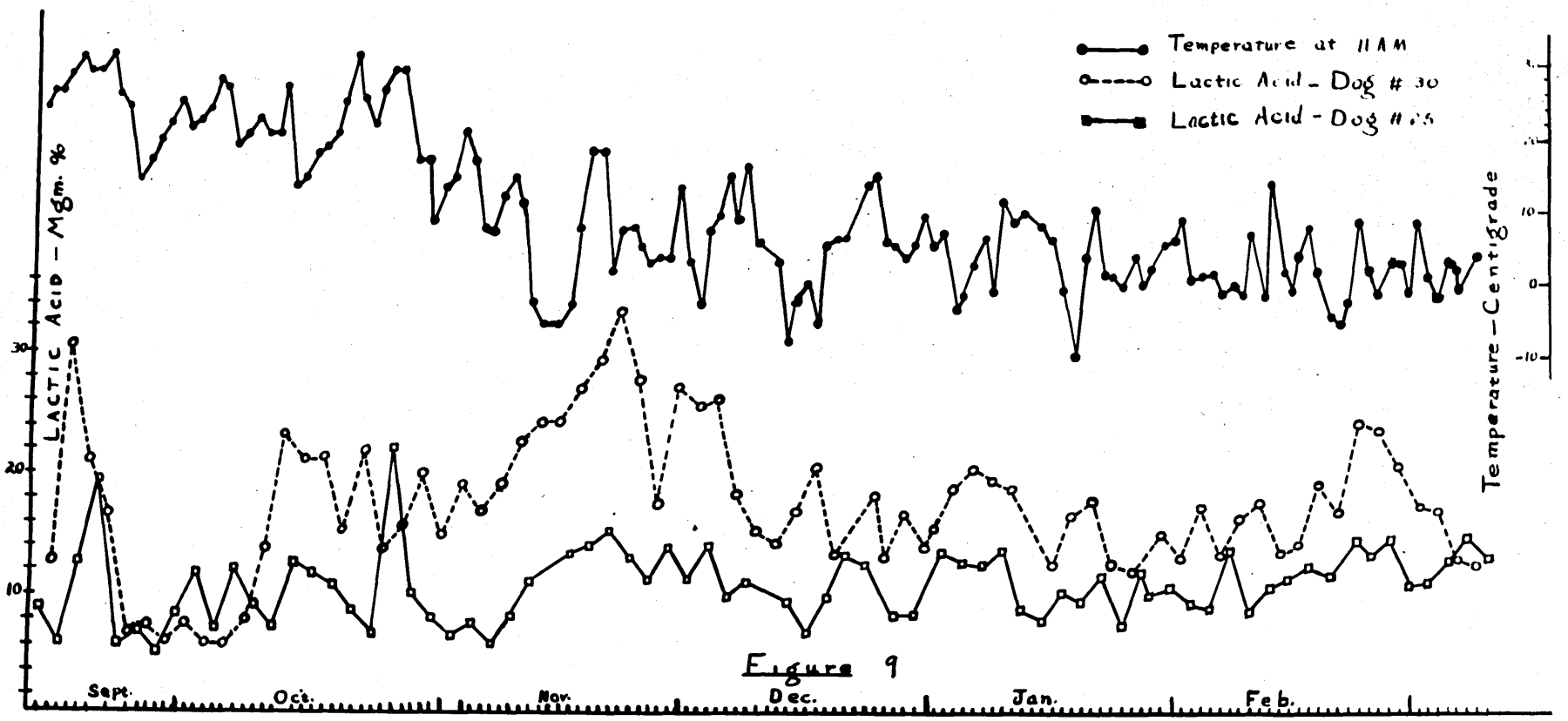
Temperature at 11 A M
Lactic Acid - Dog # 30
Lactic Acid - Dog # 35
LACTIC ACID - Mgm. %
Temperature - Centigrade
30
20
10
10
0
-10
Sept.
Oct.
Nov.
Dec.
Jan.
Feb.

Figure 9

therefore the increase may seem more abrupt than it probably really is.

Even though there is a great variation from one sampling day to another, yet this variation is no greater than is the variation observed when several successive samples are taken from a dog during a twenty-four hour period.

In addition to the greater variability, dog number 30 consistently contained a higher concentration of lactic acid in her blood than did number 25. This is well shown in Table XIX which gives the daily average value in each month.

If the general trends of the two curves are followed, a rather definite similarity in crests and troughs can be distinguished. The closest agreement, in this respect, is observed during September and the first half of October, where there is quite close agreement of even the sharp peaks. Nowhere else do these spikes agree so completely. The more extended swells, as from November 2-21, also show more or less of a similarity. In most of these more extended swells there is a compounding of progressively increasing or decreasing values making up the ascending or descending limbs respectively. There is no semblance of a plateau at any time during the six months' period, which should be expected if lactic acid concentration is stable in nature.

It is obvious that there is no relation between the blood lactic acid changes represented and the absolute

daily temperature changes. Neither is there a direct relation between average lactic acid values for a given month and the average 11 A.M. temperature during the month. However, examination of Figure 10 shows a close correlation between average monthly lactic acid values and a variable which we have chosen to designate the "average degree difference from the monthly mean." This quantity and the statistical mean deviation are identical. It is a measure of the dispersion of the daily 11 A.M. temperatures from the mean 11 A.M. temperature for a given month. Surprisingly close agreement is observed between these two variables for most of the experimental period. There is some divergence during February, but in the main the agreement is significant.

## DISCUSSION

A general trend in the same direction with respect to both dogs is observed. This similarity indicates that the changes in lactic acid are brought about by some common agent or agents. But any such blood lactic acid response may be modified, no doubt, by a multiplicity of factors.

At first glance it seems that there is no connection between blood lactic acid and variation in temperature. This does not agree with the observations of Truka-Tuzson (1940) who obtained a direct relation between monthly mean temperature and mean lactic acid in man. Of course,

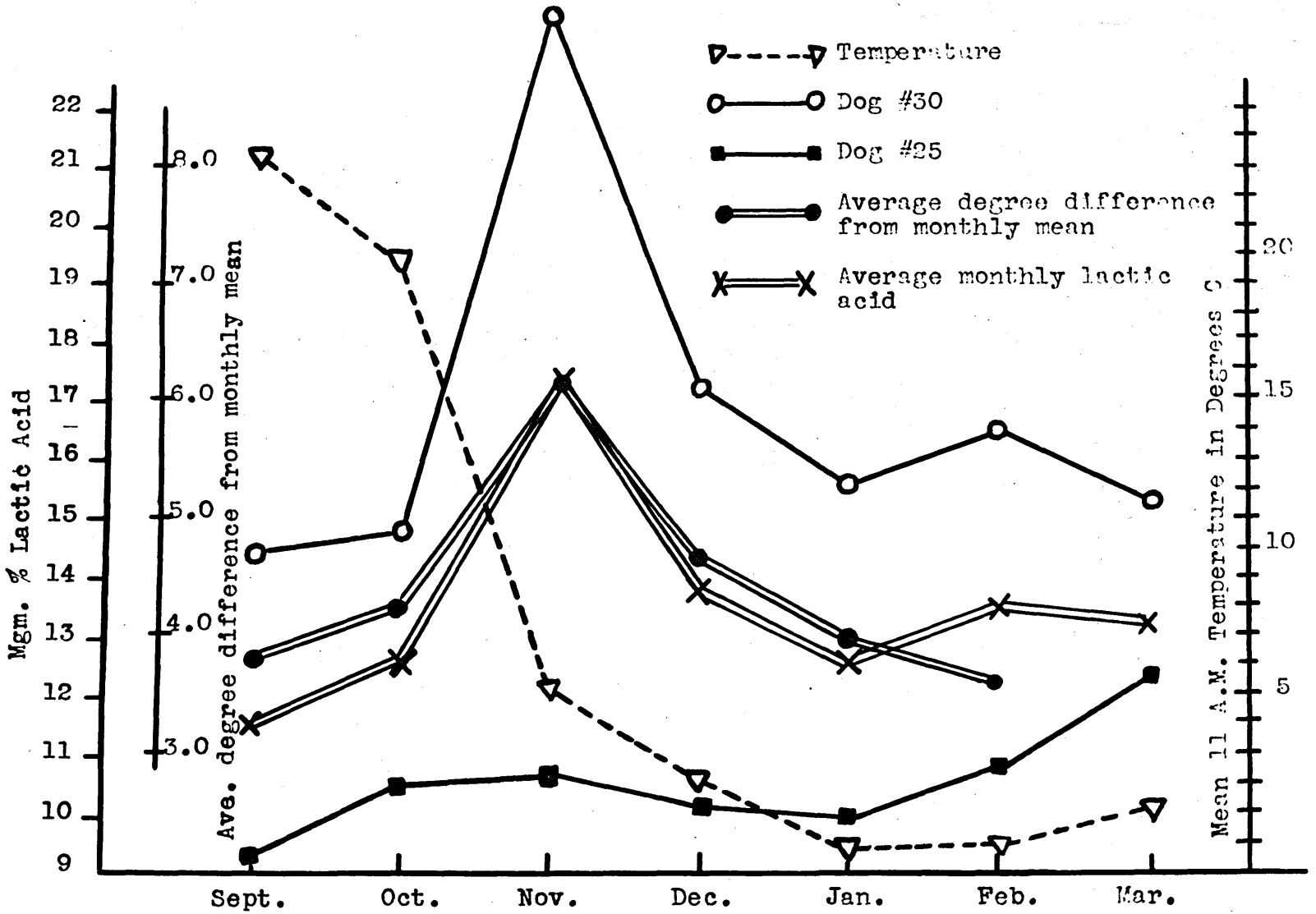


Figure 10. Average values for blood lactic acid and temperature.

there may be a species difference here. If such a difference can not be shown, it may be that the number of experimental animals in my series is too small to bring out such a relationship. It is not at all clear from Truka-Tuzson's work whether he used twenty to thirty different people each month or the same persons month by month. If he used the former, sufficient individual differences might be included to give such results, although it seems highly unlikely that such might occur. At any rate, our results tend to vary in the opposite direction from this author's.

Table XX and Figure 10 show that our results are closely related to degree of variation of the temperature from a central mean figure. This variation is expressed by using the mean deviation for a given month. Such behavior is interpreted to mean that it is not necessarily the variation from one day to the next that results in a lactic acid change, but more probably the temperature fluctuation around a central point.

Perhaps, too, a total temperature change may influence the lactic acid, since the February lactic acid values were higher for both dogs than the mean deviation would seem to warrant. The mean temperature during this month was the lowest of the whole period. Therefore, it may be that, in addition to the degree of variation as a factor, a large temperature change of continuous nature is an additional factor.

With respect to the March values, disagreement here is seen. It is felt, however, that too few values are represented to base any definite conclusions.

It may be that the mechanism operating to bring about such responses is of a nervous nature, involving the structures usually acting in temperature regulation. Yamada (1940) has shown that, in rabbits, after bilateral splanchnicotomy there is not obtained the usual hyperlactacidemia following temperature changes. This would indicate a possible involvement of the adrenal medulla, the secretion of which has been shown to influence the blood lactic acid level (Cori, 1925).

## SUMMARY

1. Blood lactic acid in dogs does not seem to vary with the day by day fluctuation in 11 A.M. temperatures.

2. No direct relation can be shown between monthly mean temperature and monthly mean blood lactic acid.

3. A close correlation does exist between monthly mean lactic acid and temperature mean deviation.

4. It is suggested that a nervous mechanism may be responsible for the regulation of these variations, since other work has shown, in the rabbit at least, that bilateral splnachnicotomy can check lactic acid production following large temperature changes.

## TABLE XVIII

### COMPLETE DATA FOR SEASONAL TEMPERATURES AND BLOOD LACTIC ACID.

| Dog #25 | | | Dog #30 | | |
|---|---|---|---|---|---|
| Date | Mgm.% Lactic Acid | Centigrade Temperature at 11 A.M. | Date | Mgm.% Lactic Acid | Centigrade Temp. at 11 A.M. |
| Sept. | | | Sept. | | |
| 13 | 8.91 | 23.3 | 14 | 12.47 | 25.0 |
| 16 | 6.04 | 24.4 | 17 | 30.60 | 27.8 |
| 18 | 12.69 | 29.4 | 19 | 21.42 | 27.8 |
| 20 | 19.11 | 27.8 | 21 | 16.33 | 28.9 |
| 23 | 5.94 | 24.4 | 24 | 6.63 | 22.2 |
| 25 | 6.93 | 13.3 | 26 | 7.13 | 15.6 |
| 27 | 5.74 | 17.8 | 28 | 6.14 | 20.0 |
| 30 | 8.32 | 23.3 | | | |
| Oct. | | | Oct. | | |
| 2 | 11.19 | 21.1 | 1 | 7.72 | 19.4 |
| 4 | 7.43 | 26.1 | 3 | 5.94 | 22.2 |
| 7 | 11.48 | 16.7 | 5 | 5.94 | 25.6 |
| 9 | 8.91 | 21.1 | 8 | 7.92 | 18.9 |
| 11 | 7.32 | 18.9 | 10 | 13.66 | 18.9 |
| 14 | 12.47 | 11.7 | 12 | 22.97 | 25.6 |
| 16 | 11.98 | 16.1 | 15 | 20.96 | 13.3 |
| 18 | 10.69 | 18.9 | 17 | 21.38 | 17.2 |
| 21 | 8.51 | 29.4 | 19 | 14.85 | 23.3 |
| 23 | 6.73 | 20.6 | 22 | 21.58 | 23.9 |
| 25 | 21.78 | 25.6 | 24 | 13.36 | 25.0 |
| 28 | 9.84 | 15.6 | 26 | 15.64 | 25.0 |
| 30 | 7.92 | 6.1 | 29 | 19.20 | 15.6 |
| | | | 31 | 14.85 | 11.7 |
| Nov. | | | Nov. | | |
| 1 | 6.33 | 13.3 | 2 | 18.41 | 19.4 |
| 4 | 7.62 | 15.6 | 5 | 16.63 | 5.6 |
| 6 | 5.64* | 5.0 | 7 | 18.71* | 10.0 |
| 8 | 7.92 | 12.8 | 9 | 22.17 | 8.9 |
| 11 | 10.69 | -4.4 | 12 | 23.96 | -7.8 |
| 15 | 12.87 | -5.0 | 14 | 23.96 | -7.8 |
| 18 | 13.36 | 16.1 | 16 | 26.93 | 5.6 |
| 20 | 14.85 | 2.2 | 19 | 28.91* | 16.1 |
| 22 | 12.67 | 5.6 | 21 | 33.26 | 5.0 |
| 25 | 10.79 | 0.6 | 23 | 27.32 | 2.8 |
| 27 | 13.46 | 1.1 | 26 | 17.03 | 1.1 |
| | | | 30 | 26.33 | 10.6 |

* No duplicate

TABLE XVIII, cont'd.

COMPLETE DATA FOR SEASONAL TEMPERATURES AND BLOOD LACTIC ACID.

| Dog #25 | | | Dog #30 | | |
|---|---|---|---|---|---|
| Date | Mgm.% Lactic Acid | Centigrade Temperature at 11 A.M. | Date | Mgm.% Lactic Acid | Centigrade Temp. at 11 A.M. |
| Dec. | | | Dec. | | |
| 2 | 10.93 | 0.6 | 3 | 25.14* | -5.7 |
| 4 | 13.66 | 4.4 | 5 | 25.54* | 7.2 |
| 6 | 9.50* | 12.2 | 7 | 17.82 | 6.7 |
| 9 | 10.69* | 13.9 | 10 | 14.85 | 3.3 |
| 13 | 8.75 | -9.4 | 12 | 13.56* | 0.6 |
| 16 | 6.53 | -2.2 | 14 | 16.4* | -5.6 |
| 18 | 10.9 | 2.2 | 17 | 19.8 | -7.2 |
| 20 | 12.5 | 3.3 | 19 | 12.9 | 3.3 |
| 23 | 11.7 | 11.1 | 24 | 17.6 | 12.2 |
| 27 | 7.92 | 2.2 | 26 | 12.5 | 3.3 |
| 30 | 7.92 | 2.8 | 28 | 16.4* | 1.1 |
| | | | 31 | 13.1 | 6.1 |
| Jan. | | | Jan. | | |
| 3 | 13.3* | 3.9 | 2 | 14.8 | 1.7 |
| 6 | 12.0 | -4.4 | 4 | 18.0 | -5.6 |
| 8 | 11.7 | 3.9 | 7 | 19.8* | -1.1 |
| 10 | 13.1 | 8.3 | 9 | 18.8 | -2.2 |
| 13 | 7.9 | 6.7 | 11 | 18.0 | 5.6 |
| 15 | 7.3 | 5.0 | 16 | 11.7 | 2.8 |
| 17 | 9.1 | -4.5 | 18 | 15.8* | -12.0 |
| 20 | 8.3 | 1.1 | 21 | 17.2 | 7.2 |
| 22 | 10.5 | -1.6 | 23 | 11.7 | -2.2 |
| 24 | 6.93 | -3.3 | 25 | 11.1 | 0.5 |
| 27 | 10.7 | -3.3 | 30 | 14.3 | 2.2 |
| 29 | 9.1 | -1.6 | | | |
| 31 | 9.5 | 4.0 | | | |

*No duplicate

TABLE XVIII, cont'd.

COMPLETE DATA FOR SEASONAL TEMPERATURES AND BLOOD LACTIC ACID.

| Dog #25 | | | Dog #30 | | |
|---|---|---|---|---|---|
| Date | Mgm.% Lactic Acid | Centigrade Temperature at 11 A.M. | Date | Mgm.% Lactic Acid | Centigrade Temp. at 11 A.M. |
| Feb. | | | Feb. | | |
| 3 | 8.7 | -2.2 | 1 | 12.1 | 6.1 |
| 5 | 7.9 | 1.6 | 4 | 16.2 | 1.1 |
| 7 | 13.3 | -3.3 | 6 | 12.1 | -4.4 |
| 10 | 7.7 | 3.9 | 8 | 15.5 | -4.4 |
| 12 | 9.9 | 12.2 | 11 | 16.6 | 4.4 |
| 14 | 10.1 | -2.2 | 13 | 12.3 | .0 |
| 17 | 11.5* | 6.0 | 15 | 13.4 | 1.7 |
| 19 | 10.9 | -6.6 | 18 | 18.6* | -1.6 |
| 21 | 13.7* | -4.0 | 20 | 16.0 | -7.2 |
| 24 | 12.5 | 0.55 | 22 | 22.6 | -6.0 |
| 26 | 13.5 | .0 | 25 | 22.2 | -3.3 |
| 28 | 9.5 | -3.3 | 27 | 19.3* | .0 |
| Mar. | | | Mar. | | |
| 3 | 10.1 | .0 | 1 | 16.43* | 5.0 |
| 5 | 12.1* | 2.2 | 4 | 16.23* | -1.7 |
| 7 | 14.2* | .0 | 6 | 12.3* | .0 |
| 10 | 12.5 | 1.7 | 8 | 12.1* | 1.7 |

* No duplicate

TABLE XIX

SUMMARY BY MONTHS

| Mean 11 AM Temp. Centi-grade | Mean Daily Mgm.% Lactic Acid | Total Mgm.% Lactic Acid | Number of Sam-ples | Month | Number of Sam-ples | Total Mgm.% Lactic Acid | Mean Daily Mgm.% Lactic Acid | Mean 11 AM Temp. Centi-grade |
|---|---|---|---|---|---|---|---|---|
| | Dog #25 | | | | | Dog #30 | | |
| 23.9 | 14.39 | 100.72 | 7 | Sept. | 8 | 73.68 | 9.21 | 22.9 |
| 20.4 | 14.71 | 205.97 | 14 | Oct. | 13 | 136.25 | 10.48 | 19.0 |
| 5.8 | 23.63 | 283.62 | 12 | Nov. | 11 | 116.20 | 10.56 | 5.7 |
| 2.1 | 17.13 | 205.61 | 12 | Dec. | 11 | 111.00 | 10.09 | 3.7 |
| -0.28 | 15.56 | 171.20 | 11 | Jan. | 13 | 129.43 | 9.95 | 1.1 |
| -0.73 | 16.41 | 196.90 | 12 | Feb. | 12 | 129.20 | 10.76 | 0.26 |
| 1.1 | 14.26 | 57.06 | 4 | Mar. | 4 | 48.90 | 12.20 | 0.97 |

TABLE XX

AVERAGE MONTHLY LACTIC ACID AND

TEMPERATURE MEAN DEVIATION VALUES.

| | Average mgm.% Lactic acid per month for dogs #25 and #30 | Average degree difference from monthly mean (centigrade) |
|---|---|---|
| Sept. | 11.62 | 3.80 |
| Oct. | 12.68 | 4.25 |
| Nov. | 17.38 | 6.11 |
| Dec. | 13.77 | 4.70 |
| Jan. | 12.52 | 3.95 |
| Feb. | 13.59 | 3.58 |
| Mar. | 13.24 | 1.47 |

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