NOTES

Chapter 1, Note 1. Subnuclear Organization

The analysis of the available data concerning subnuclear particles shows that while an unsuspected number of different particles are progressively discovered, no satisfactory relationship between them—from the point of view of the organization—can be established. The recognition of a pattern concerning their organizational relationship would fill an important gap in the knowledge of this entire field. The fact that the same pattern governs the organization in general, from atomic nuclei up, has induced us to attempt through an extrapolation, to search it for the subnuclear realm.

As seen above, the study of the organization has permitted us to define the following concepts as characteristic for the organizational pattern:

1) All the entities in nature can be identified by their place in a hierarchic organization, in which the entities are connected through a superior-inferior relationship. An entity enters in the formation of other entities which are considered "superior" to it, and is formed by entities which are hierarchically "inferior" to it.

2) Each entity is formed by a principal and a secondary part, the principal part being represented by entities hierarchically inferior to it, while the secondary part, by elements taken from the immediate environment in which the entities forming the principal part have existed.

3) Entities with similar principal parts belong to a same level. In the hierarchic progression, there are entities of the same level which are grouped together to constitute the principal part of an entity of a level immediately superior.

4) From the energetic point of view, the principal part in the organization of each entity appears more positive than the secondary part to which it is bound.

5) The hierarchic progression of the organization from one entity to that immediately superior to it, is made through two processes with two different intervening forces. Forces of columbian nature bring and keep the electrostatically opposite principal and secondary parts together. A new entity appears however only when quantum forces intervene, organizing the relationship of the two constituents and especially their reciprocal movement. The immediate aim of this organization is to prevent a reciprocal total annihilation of the two parts, positive principal and negative secondary which would occur if the electrostatic forces alone would be present.

6) In the hierarchic progressive development of the organization for

each passage from one level to the immediately superior, these two kinds of forces—electrostatic and quantum—were seen to intervene alternatively. The fulfillment of one force is seen to induce the appearance of the other. From an energetic point of view, an entity will appear inactive when its electrostatic forces are fulfilled but with quantum forces present, or energetically active with quantum forces fulfilled and the electrostatic forces present. The example of atoms and ions is a typical illustration of this relationship for the atom level.

As a work hypothesis, we tried to apply the above schematically presented concept of hierarchic organization, to the subnuclear realm. It is only by using analogies that such an attempt can be made. The scarce data available seem to confirm however, this view. According to it, the electron and positron would represent the lowest entities of the subnuclear realm with the smallest mass and opposite charge. If these two corpuscles, when attracted, encounter one another, they will annihilate each other with liberation of two photons. This annihilation is prevented however, although the two corpuscles remain bound together through their electrostatic forces by the intervention of quantum forces organizing their reciprocal movements. The result is a new entity, of a level immediately superior to the positron, in which the electron is kept electrostatically bound to the positron but kept into an orbital movement. Hypothetically, it can even be conceived that through differences in the resulting movement, more than one solution would exist.

Residual Charge

Due to the intervention of this movement, the resulting electrostatic neutralization between positron and electron is incomplete. A "residual" positive charge would characterize the new entity. This charge alone would not be sufficient to keep another electron by neutralizing its charge. However several such entities grouped together can have the sum of their "residual positive charge" such as to be compensated by a new electron. The two electrostatic forces, that of the group of entities and that of the new electron will keep these two parts together, while the quantum forces will again organize the movement of this new added electron, preventing this time again the annihilation of these two parts. A new level, this time of the third order, is thus realized. It is easy to conceive that several solutions can exist for each case, since the sum of the residual positive charges does not correspond exactly to that of the negative electrons. Several solutions appear thus possible. Besides this in which a small group of entities would be compensated by one electron, a higher number of entities would be kept together with the sum of their residual charges approaching that of two or more electrons. For each level, several such solutions are conceivable. With the progressive passage toward higher levels, the number of the solutions increases.

The fact that the two electrostatic positive and negative parts of the entities do not compensate perfectly, leads to the possibility that the compensation takes place either with an excess or lack of negative charge.

The difference is induced by an additional electron. An entity with a positive or negative charge would result. With one or the other charge prevailing, two energetically active, positive or negative forms, differing by the mass of an electron exist, for each entity. The analysis confirms this existence.

According to the hierarchic organization, the different particles of the subnuclear realm can be separated in various levels. Promezons, mezons, protons would represent such levels—each one with respective neutral, positive and negative entities. The same as for the higher levels—atoms and molecules—the different entities forming each of the subnuclear levels will differ through the number of the entities entering in the principal parts. A systematization of the subnuclear realm on this basis can be confirmed by the fact that the different entities of a level represent sums or multiples of the entities of their inferior level in the same way that the different nuclei represent multiples of entities of the protonic level protons and neutrons or the different molecule multiple of entities of the atomic level.

In the progression of the hierarchic organization, it is seen that the passage from one level to the other results in an exponential increase in the numbers of the kinds of entities (from around 100 different atoms it passes to around 100,000 different kinds of molecules, to millions of kinds of genes and trillions of individuals. This fact supposes that the number of the existing particles decrease with each inferior level, in the subnuclear realm, to arrive to two—positron and electron—at the bottom of this realm.

Chapter 2, Note 1. C-N-C-N

As far back as 1905, Kossel has had indicated the existence for the important alkaline aminoacids, arginine and histidine, of the C-N-C-N group, found also in the nitrogen containing bases of nucleic acid. The hypothesis which we advance that this C-N-C-N group would represent the starting point of the biological realm itself, can surely be subject to discussion.

Progressively more evidence is being obtained that important organic compounds can appear from the constituents of the atmosphere itself, under the influence of electrical discharges or of ionizing irradiation. While Henriet (268) was the first to show that formic acid is present in rainwater, it was Loew (269) who obtained glycine from the constituents of the atmosphere submitted to electrical discharges. By utilizing, under the same condition, mixtures similar to those considered to have been present at the time when life is supposed to have started, Miller obtained, through electrical discharges, many amino acids and other substances especially glycine and formic acid (270, 271). Miller's results were extensively confirmed (272 to 279).

The irradiation of the mixtures of gases considered present in the atmosphere millions of years ago has led to the synthesis of many other substances such as formic, acetic, propionic, succinic and even tricarboxilic acids (280, 287). From these, we consider of especial importance the

members with a second nitrogen group far in the molecule, as diamino-succinic acid, iminodiacetic or iminoacetic-propionic acid.

The synthesis of the strongly positive C-N-C-N group which we consider as the starting point of the biological realm, seems thus to have taken place rather under the influence of radiation. This fact appears especially important since it would relate more directly the beginning of the biological realm to the intervention of the radioactive elements, which according to our systematization of the elements, form the period which corresponds to the lowest levels of the hierarchic organization. *(See Chapters 2 and 5)*

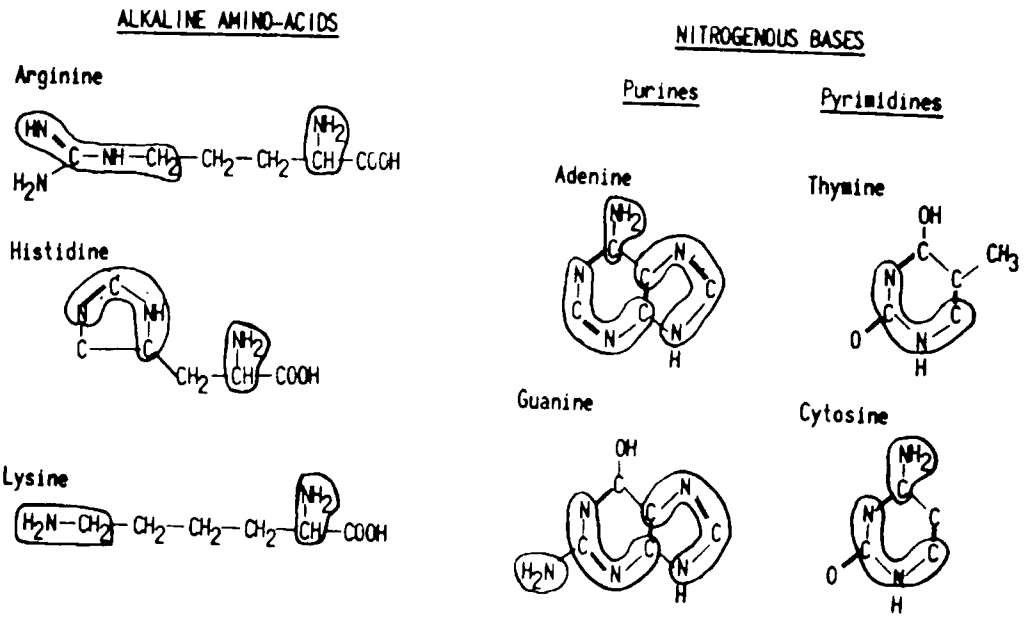

FIG. 201. The NH_2 and C-N-C-N groups appear as entities taking part in the formation of alkaline amino-acids as well as of nitrogenous bases. The bond to a chain having an amino acid group in the first case, results in a new entity—an alkaline amino acid—which polymerizes through the amino-acid group. Through the alkaline group it conserves its positive electrical character. In the nitrogenous bases, the C-N-C-N group is part of the cycle. Bound to phosphoric acid, the results are acid entities with negative charges.

The further evolution of the C-N-C-N formation seems to have taken place in two directions—one in which one or two such groups have formed a cycle and given rise to the nitrogenous bases, purines and pyrimidines, and the other in which this energetic group has bound an aliphatic amino acid chain, this last probably originated under the influence of electrical discharges. The two principal alkaline amino acids, arginine and histidine, have thus appeared. *(Fig. 201)* The double capacity of the alkaline amino-acids, to bond other amino-acids through their amino-acid groups and thus to form polymers, and to bond acid substances through their alkaline polar groups and make new hierarchic entities, has given these substances their peculiar organizational role. C-N-C-N, alkaline amino-acids and histones

(or in fish, the protamins) would thus represent the first hierarchic steps in the progression of the biological series.

A few words should be said about lysine, the alkaline amino-acid with an amino group as alkaline terminal group. Although together with the other alkaline amino-acids, it enters in the formation of histones, it seems to have another important biological role, that of an agent intervening in the metabolism of lipids.

Chapter 2, Note 2. **Distribution of Potassium and Sodium**

As mentioned above, potassium is the cation of the cytoplasm, the secondary part of cells, while sodium is the cation of the secondary part of the metazoic compartment, that is of the fluids of this compartment. According to the view presented above, the peculiar distribution of these two cations in the biological realm results from their similar distribution in the environments from which these respective secondary parts, cellular and metazoic, are considered to have been derived. As we related the cytoplasm to mud, respectively to the lithosphere, and the fluids of the metazoic compartment to the sea, we looked for a confirmation of this view in the comparison between the amount of these cations in the two biological compartments and in the two environments which we consider to correspond to them.

Although potassium and sodium are in almost equal amounts in the general constitution of the earth's crust, potassium is found almost entirely in the solid parts while sodium forms the principal constituent of the salts of the fluid part of the earth. The distribution of potassium found between cells and extracellular fluids seems very near to that which exists between lithosphere and hydrosphere. Potassium is found in a proportion of 2.46% of the lithosphere and only in 0.04% of the hydrosphere (201). The ratio of these respective concentrations corresponds to a $^1/_{61}$ value. This seems near enough to the ratio found in biology. While the extracellular potassium represents only 5 mEq per liter, with a total of 70 mEq (2.7 gm.) for a normal body, the intracellular part corresponds to 115 mEq per liter of cells, with a total of 4,000 mEq (160 gm.) for the body (202). The ratio of $^1/_{59}$ for total extracellular and intracellular is accepted today although generally considered too high when compared with the previous data given years ago by Shohl (265). This value of $^1/_{59}$ appears impressively near the ratio of $^1/_{61}$ found in the comparison of the potassium content of the lithosphere with that of the hydrosphere.

A similar resemblance is encountered when comparing the proportion between sodium and potassium in two fluids: the interstitial fluid of the body and of the sea. The two ratios of these elements appear close enough. For instance, the Atlantic Ocean has 10.464 gm. of sodium per thousand and 0.725 gr. per thousand of potassium, while the Pacific Ocean has 10.233 gr. per thousand of sodium and 0.634 gr. per thousand of sodium (266). The ratios between sodium and potassium are respectively 14 and 16. In the blood serum, the ratio is 16 when the average is considered as

320 mg.% of Na, and 20 mg.% of K. Table XXV shows these comparative values.

TABLE XXV

(a) Comparison between the Extracellular and Intracellular Potassium and the amount present in Hydrosphere and Lithosphere.

		Ratio
Potassium extracellular-total body	2.7 gm.)	1/59
Potassium intracellular-total body	160 gm.)	
Potassium in the hydrosphere	0.04%)	1/61
Potassium in the lithosphere	2.46%)	

(b) Potassium and Sodium in the sea and in the body fluids.

	Na	*K*	*Ratio*
Atlantic Ocean	10.464 gr. 0/00	0.725 gr. 0/00	14.4
Pacific Ocean	10.233 gr. 0/00	0.634 gr. 0/00	16.0
Blood Serum	320 mgr. 0/0	20 mgr. 0/0	16.0

These values seem to bind the distribution of K and Na, seen between the cellular and metazoic compartments, to that which exists between the environments from which we consider these respective secondary parts to have been derived.

Chapter 2, Note 3. **Social Hierarchic Organization**

The organization of nature with the characteristic hierarchic structure so evident in the biological realm, has suggested a similar structure in social organization.

All the factors which were seen to characterize hierarchic organization appear clearly in an analysis of social organization. *(Fig. 202)* The entity, immediately above that of the individual, is the family. Here, parents and children—as a grouping of entities of the same level form the principal part. The secondary part is made up of elements of the immediate environment, which are kept organized around this principal part, and as such are integrated in the new entity, the family. Housing, goods, even psychological factors, ideas and habits, characterize these added factors. A boundary formation is often much more visible than expected. Living quarters and common possessions are well delineated, and characterize the family. As expected, most of them are not considered to belong to an individual but the family as entity. "This is family property" is a common expression.

Almost always, numerous families are grouped in nearby dwellings, although this fact alone does not lead to the immediate superior entity, the community. When the group of families organizes together and limits certain possessions taken from the environment, as common to the group, the entity "community" appears. The principal part is made up of the group of families, the secondary by the material and even moral goods which are attached to the group of families in common. The community has prop-

erties which belong only to the community—streets, for example—as it has, by definition, a boundary. The limits of these social entities are well defined and these three factors—principal part, secondary added part from the environment and boundary—characterize these entities as they characterize the entities in the entire biological realm. The same pattern applies for the county where groups of communities form the principal part, and

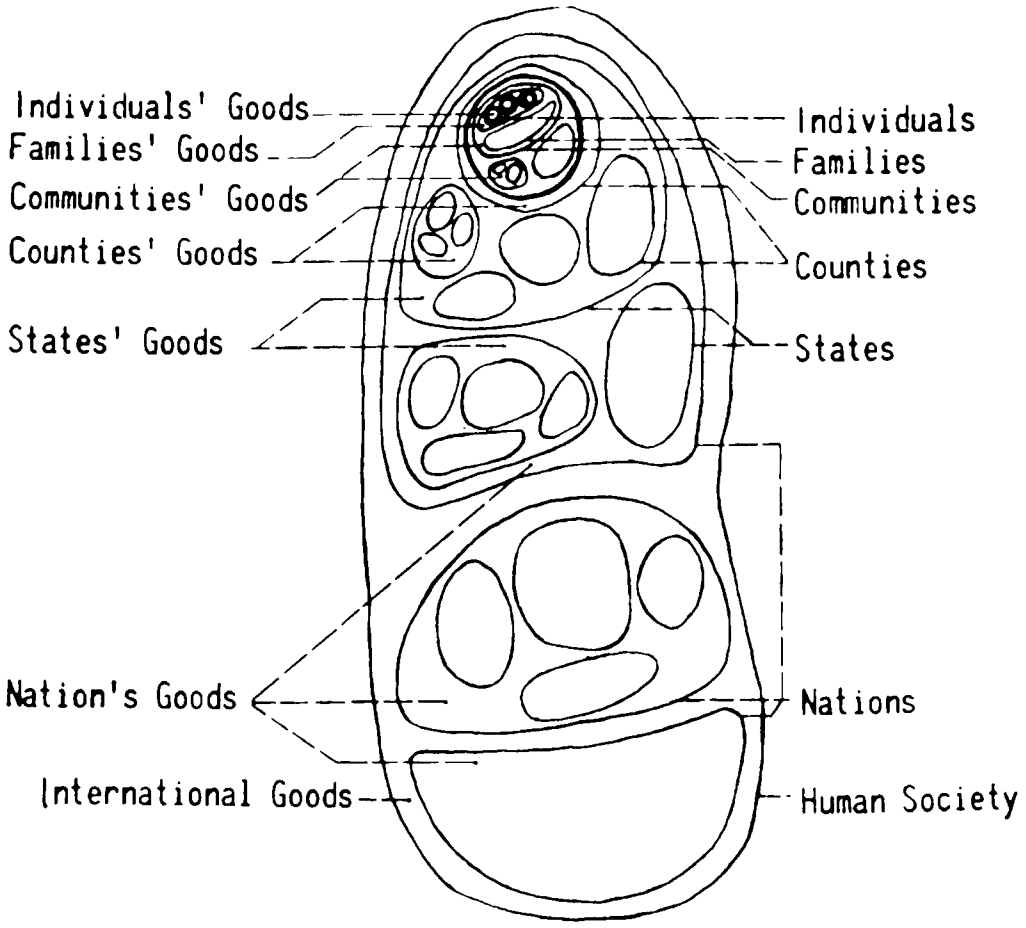

FIG. 202. The *social hierarchic organization* follows the same pattern as the organization of matter or of the biological realm. Each entity results from the bond of a group of lower entities with a secondary part taken from the environment and limited by a proper boundary.

proper parts taken from the environment and common only to this new entity form the secondary part. This entity also is defined through its boundary. It is easy to see how through the same hierarchic pattern we pass from counties to states, nations, hemispheres and world which represent successively higher hierarchic entities. It is interesting to see how, in each one of these social entities, the same manifestations which we have found to characterize the biological entities also exist. The relationship between entities and especially many of their functions shows that the

social entities are not artificial mental concepts, but are the result of the intervention of the same forces in which heterotropic organization opposes the lawless homotropy. It has appeared interesting to see how much of the knowledge of the physiological and especially the pathological manifestations of the lower entities, we can apply to understand manifestations occurring at the social hierarchic levels.

Under this aspect, sociology finds a new basis not only for the analysis of many of its problems, but can have an insight to how nature, through its own organization, has tried and often succeeded in resolving problems. With the concept of unity in all organization, from subatomic to social entities, we can understand how the evolution of the environment, represented by material and intellectual goods, can produce changes in social entities. The concept of higher social entities, organized so as to conserve the characteristics of the lowest social entities, gives a new aspect to the relationship between individual, family and society. A science of social physiology can be created by systematizing hierarchic social entities much as we did for entities in the biological realm. The same approach can be applied to social pathology and social therapy as well. Such an approach, will be the subject of other presentations.

Chapter 3, Note 1. **Precancerous Lesions**

Precancerous lesions were identified especially in cases in which cancerous lesions were induced and where a manifest polycentricity of lesions was present. (203) Polycentric lesions permit us to study the entire successive changes from normal to invasive cancer.

Induction of cancer in the stomach of rats through carcinogens and detergents (204) has furnished excellent material for such study; it has also permitted us to characterize the specific changes. Among cells which appear grossly normal, there are some in which certain morphological characteristics of the nucleus, notably size and form, appear abnormal. The existence of an anomaly is much more evident when the cell divides. It may be limited to just a few chromosomes which are abnormal in their dimension and form. This chromosomial abnormality appears still more evident when compared to cells in mitosis in controls with normal mucous membranes. (309)

Chapter 3, Note 2. **Non-invasive Cancer**

We have emphasized the character of the cytoplasm of the cells in non-invasive cancer. The nuclei show a number of changes which, together rather than separately characterize a cancerous entity: an irregular shape of the nucleus with a manifest increase in size; a sharp nuclear border formed by a dark pigmented nuclear membrane having fine chromatine particles; a hyperchromatism with clumps of chromatin separated in bizarre, irregular fashion; and an uneven, irregular distribution of these chromatin clumps, concentrated near the nuclear membrane. Also often

encountered is the presence of one or more irregular enlarged nucleoli, with a distinct nucleolar border and especially with a manifest acidophilic staining.

In the non-invasive cancer, all these nuclear anomalies contrast with the relatively normal cytoplasm, which has not only an acidophilic reaction —colored in orange with Papanicolau's trichromic staining—but also a well-defined cell membrane with fairly clear cell border. The size of the cytoplasm—compared to other cells—is normal, although the nuclear-cytoplasmic ratio is increased due to the big nucleus. Due to the character of the cytoplasm, these cells were called the "third type differentiated cells" by Graham. (205) We emphasized the "normal" aspect of the cytoplasm of these non-invasive cells, in contrast to the invasive cells where the abnormality includes both the nucleus and the cytoplasm. This explains why most of the invasive cells have little cytoplasm, an indistinct cellular border and a basophil cytoplasmic staining. (206) But besides these cells with totally abnormal cytoplasm, there are some invasive cells with an apparently differentiated cytoplasm. Although their staining is orthochromatic, their cytoplasm shows marked abnormality in form. The tadpole cells found in the exfoliative cytology in epidermoid carcinoma, (207) or the fiber cells (208) with abnormally long fibrillar cytoplasm revealed in other forms of invasive cancer indicate a participation of the cytoplasm in the abnormality. The cells found in so-called "Bukhead's disease" with minimal abnormal cytoplasm thus appears to be at the boundary between non-invasive and invasive cancerous cells.

Chapter 3, Note 3. **Abnormal Amino Acids**

We have seen above how the concept of hierarchic organization brought us to consider the alkaline amino acids and the histones which they form as one of the first members of the biological realm. Anomalies can be conceived to result from a process of resonance which occurs constantly on a statistical basis. As work hypothesis, we consider such resonance entities as corresponding to these abnormal forms, which in hierarchic development would lead to cancerous entities.

The naturally existing levorotatory alkaline amino acids represent the constituents which, through their number and role in further organization, represent normal entities. Opposed to them, the dextrorotatory alkaline amino acids would represent abnormal entities. Their existence and their role has made the object of many discussions without, however, bringing sufficient light to this problem. The constant existence in the body of specific enzymes against these dextrorotatory amino acids in spite of the fact that they are not recognizable analytically, indicates a certain defense against them. The concept of their appearance as a resonance phenomenon would explain easily this occurrence. Dextrorotatory amino acids, although abnormal for the organism, exist in practically all individuals as a resonance form, but they are not able to develop—or develop in extremely reduced form—because of the enzymes which attack them. They are, how-

ever, able to develop the lowest levels of cancerous entities as they are recognized to exist practically in all normal individuals particularly after a certain age. There are these considerations which lead us to believe that it is the dextrorotatory resonance form of alkaline amino acids which represent the abnormal entities at the lower levels.

Chapter 4, Note 1. **Physiological and Pathological Pain**

Physiological Pain

Physiological pain may be defined as a specific sensorial sensation induced in normal tissues when external stimuli are applied with sufficient intensity to endanger tissue integrity. Because pain may be induced by a wide variety of stimuli, it has not always been accepted as constituting a sensorial sensation. Considerable evidence exists, however, to indicate that physiological pain is a specific sensation, similar to the other sensorial sensations. One indication that pain is a proper sensorial sensation lies in the fact that its has its special nerve system.

Blix (5) and Goldscheider (6) found that certain areas of the skin were sensitive to painful stimuli from a pinprick while others were not. Strughold (7) demonstrated that, in various areas of skin, pain points were concentrated in varying degree. Microscopic study of areas of skin showing high aggregations of spots of specific forms of sensibility has indicated that special sensory nerve and organ structures are apparently associated with different types of sensation. Thus, Krause's corpuscles are considered as receptors for cold; Ruffini's endings and Golgi-Mazzoni corpuscles for heat; and Meissner's corpuscles, Merkel's discs and the basket endings around hair roots for touch. (8) Woolard (9) described unmyelinated, finely beaded, branched free endings as the specific nerve end organs believed to be responsible for the reception of pain impulses. Certain areas such as the cornea and the mucous membrane of the nose, which are generally considered sensitive to pain alone, have been shown to have these free endings as the characteristic nerve endings at these sites. Weddell has found only this type of end structure in areas of skin sensitive solely to pain during nerve regeneration.

That pain constitutes a specific form of sensation is further indicated by the evidence that its impulses are carried along definite nerve pathways to special centers in the thalamus. By temporary asphyxia, by cocainization, or by cooling, differential interference with conduction of the special sensations of pain, touch, heat and cold along a nerve can be produced. The existence of individuals without the sensation of pain, but with sensations of touch, cold and heat, has confirmed this view of pain as a proper sensorial sensation.

It is characteristic of the sensation of pain that it may be elicited by a wide variety of stimuli. Below the pain threshold, the incitation induces specific sensorial sensations according to the stimulus used. Above this threshold, the sensation felt is pain. When different noxious stimuli produce pain, the subject cannot distinguish the nature of the incitation. In effect,

when it is below the threshold, the incitation informs about the nature of the stimulus; above the threshold, the individual is conscious of another fact: that the stimulus is of such intensity as to endanger the integrity of the tissues. Pain thus appears to be the sensorial sensation of a specific character of stimuli—sufficient intensity to represent a danger for the tissues, and it is this which differentiates pain from the other sensorial sensations, and puts it into a special category. Pain is independent of the nature of the stimuli. By constituting a warning to the body, that its tissues are in jeopardy, physiological pain induces a general response involving brisk, rapid movements, a rise in pulse rate, and a sense of invigoration. (10)

The fact that sensorial pain results from the intensity of the external incitations has prompted investigators to study this kind of pain largely in terms of the threshold of incitation. It must be emphasized that for each stimulus there exist two thresholds, one for intensity values required to produce specific sensations, and the second for intensity needed to produce pain. There is a considerable difference, for example, between the heat intensity necessary to produce a sensation of warmth and the amount that will produce a sensation of pain.

Pathological Pain

Pathological pain differs profoundly from physiological pain. It is a psychic response to impulses originating in tissues which are abnormal either because of damage produced by external stimuli or because of inflammatory, circulatory, neoplastic, or other processes. When pain is present as a consequence of tissue damage or disease, it can no longer be considered as a warning of danger but constitutes a sign of injury.

The general response to pathological pain is totally different from the response to physiological pain. Instead of the organism being prepared for fight or flight, its efforts are directed toward placing the painful injured area, or the entire body at rest; and, to protect the painful area from further injury, the pulse rate generally slows, the blood pressure falls and often there is sweating and nausea. (10)

The local nature of the changes responsible for pathological pain has raised the problem of the several possible mechanisms of action which may intervene in inducing this pain.

1. Locally originated stimuli produced by damaged tissues themselves may act directly upon the pain end organs to induce pain impulses. Lewis has suggested that the pain associated with tissue damage is a result of the action of locally elaborated abnormal chemical substances. (10) This possibility was first considered by von Frey (11) although actually the second pain described by him was due to different rates of pain impulse transmission. Lewis (10) and his associates have studied pain in erythralgia which represents a typical form of pathological pain. (12, 13) They have shown that when skin has been injured and thus rendered hyperalgesic but not actually painful, simple arrest of circulation to this injured area may induce pain. A similar phenomenon is evident when a muscle is exercised vigorously while its circulation is arrested. If the constricting blood pressure cuff

is released, the pain that is experienced during the period of ischemia disappears, but if the cuff is reinflated, pain may recur without further exercise. In both instances, no new stimulus is required to arouse pain. It has been found that in erythralgia, neither vasodilatation nor change in skin temperature is the factor responsible for lowering the pain threshold. According to Lewis, when the circulation to the affected area is severely reduced, accumulated stable chemical substances elaborated by the damaged tissues may act directly as the pain stimulus. No definite evidence has been offered by these researchers, however, as to the chemical nature of the elaborated substances involved.

2. Local changes in damaged tissues may bring about a lowering of the nerve threshold for pain. Lewis has demonstrated the spread of the lowered threshold to nerves far beyond the site of the lesion itself. He studied the cutaneous hyperalgesia following tissue-damaging excitation of a tiny area of skin by a tapered forceps or faradic current. By producing damage in a previously anaesthetized area, he found that the local changes brought about by the damage did not produce hyperalgesia in the surrounding skin until the effects of the local anaesthetic wore off. The localized nerve changes then created a wide zone of hyperalgesia for prolonged periods. Tower (14) has presented evidence to show that the receptor end structures for pain have an arborizing rather than a plexiform arrangement, thus making unnecessary the postulation of an autonomically unidentified "nocifensor" nerve system, as proposed by Lewis, (15) to account for the type of spread of the hyperalgesia. The extent and especially the distribution of this area of hyperalgesia has clearly indicated that it is the result of a lowered threshold in the arborizing branches of the cutaneous nerve, a few branches of which were originally intensely stimulated. When a few fibers of a cutaneous nerve were directly stimulated, the same effect was observed. The findings suggested that a local tissue change lowers the threshold for pain for the nerve endings of the damaged area, and that this effect may spread through other branches of the cutaneous nerve involved as well as through larger nerve trunks so that the resultant area of hyperalgesia becomes very extensive.

3. Local changes may alter end organs ordinarily concerned with other forms of sensation in such a way that the impulses originated by them evoke the sensation of pain. Certain areas such as the appendix and the mucosa of the stomach apparently cannot, under normal circumstances, be incited to respond painfully to any form of stimulation. (16) However, in the presence of inflammation, the same stimuli may give rise to pain in these areas. The relationship between the end organs or nerves ordinarily concerned with the reception of other forms of sensation and those of pain has been considered by several authors. Weddell (17) has demonstrated that the various complex end organ structures are supplied with accessory fibers, unmyelinated and beaded, analogous to those considered to be pain receptors. Head (18) has shown in experiments on the glans penis that there may be a fusion of various sensations into a single concept and that one sensation may inhibit another. According to Feng, (19) the balance in

excitability between touch and pain receptors may be upset peripherally by liberation of a chemical substance as the result of injury.

Considerable evidence exists to indicate that pain is the most primitive form of sensation. It is possible that in the presence of pathological disturbances, through dedifferentiation complex systems for the reception and transmission of other modalities of sensation come to act as pain receptors.

Whatever the exact mechanisms may be, the findings of different investigators have led them to the conclusion that abnormal chemical substances are released from pathologically affected tissues and that these chemical substances may play an important role in the production of pathological pain.

Chapter 4, Note 2. **Blood Titrimetric Alkalinity and Urinary pH**

The important role of the kidney in regulating the acid-base balance of the blood has been described (209) and a general relationship between daily acid excretion and plasma bicarbonate has been recognized. (210) However, a consistent relationship between blood acid-base variations and urine changes has not been clearly established.

The determinations of blood pH and CO_2 combining power are the most commonly employed methods for following acid-base changes. However, they indicate only certain distinctive factors intervening in acid-base balance. The pH is a measurement of the dissociated elements in the blood and is maintained within narrow limits by the buffer mechanisms, while the CO_2 combining power is a measurement of only one of the multiple factors in the buffer system, the bicarbonate group. (211) The inconsistent relationship between variations in urinary pH and blood values indicate that changes in urinary pH depend upon factors other than the blood's dissociated substances and bicarbonate-carbonic acid buffer mechanism. The phosphates, proteins and hemoglobin are among the members of other important buffer systems that have a role in the control of the acid-base balance of the blood. *(Figs. 203, 204)*

The titrimetric alkalinity of the blood represents a measurement of the totality (reserve supply) of the substances, both dissociated and non-dissociated, that are involved in the maintenance of the acid-base balance of the blood. (212) We considered it interesting to examine the relationship between blood titrimetric alkalinity and urinary pH. Concomitant variations were compared.

Human subjects and dogs without apparent kidney dysfunction were used. Blood was obtained by venipuncture with an accurately calibrated dry syringe. After the needle was introduced into the vein and before withdrawing blood, the tourniquet was released for several minutes to avoid changes due to stasis.

Exactly 5 cc. of blood was introduced directly into a flask containing 30 cc. of a 0.001N sodium hydroxide solution. The flask was immediately closed with a rubber stopper and the mixture agitated sufficiently to assure homogeneity. If determinations were not carried out at once, flasks were

stored at 5°C and then brought to room temperature (20°C) before analysis. Fifty cc. of distilled water was used as a wash when flask contents were transferred to a beaker for titration. The total alkalinity was determined by electrometric titration to pH 7.0 against a .01N hydrochloric acid solu-

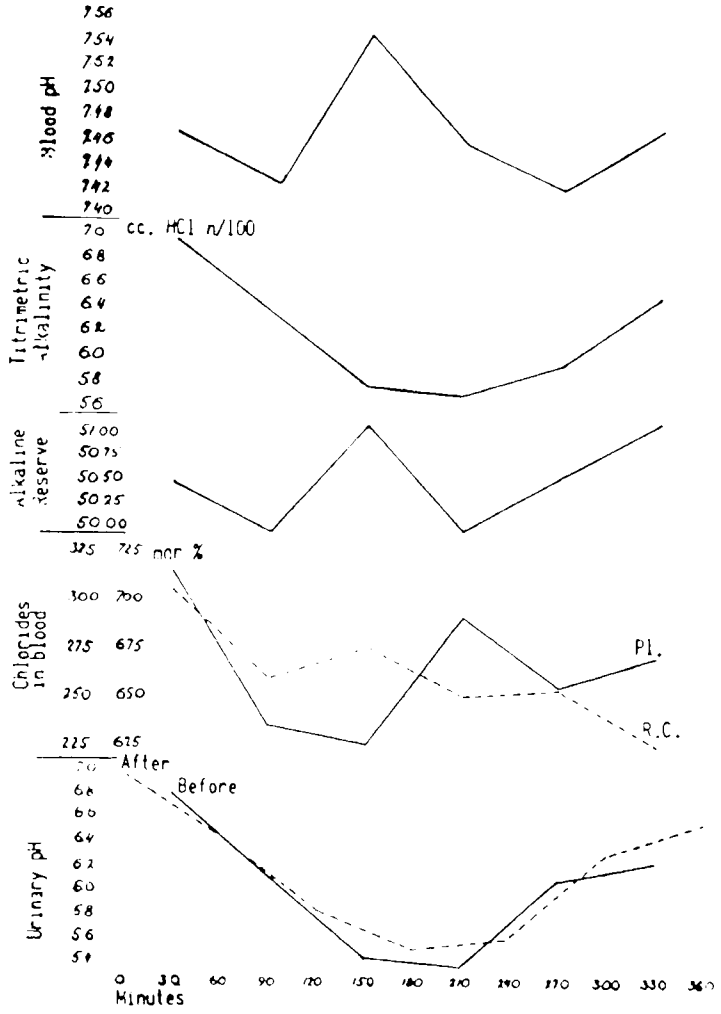

FIG. 203. The comparison between the concomitant changes seen in various blood and urine analyses concerning the *acid-base balance* of the body. It shows that the variations of the titrimetric alkalinity of the total blood are the only values which constantly parallel those of the urinary pH. (Patient with breast cancer)

tion using a Beckman pH meter, Model G, and a mechanical stirrer. Blanks of 30 cc. of the sodium hydroxide solution were stored and treated in the same manner.

Urine specimens from the human subjects were obtained through complete emptying of bladder contents by voluntary micturition. An indwelling catheter was used for dogs and some humans. Specimens were placed in containers closed with rubber stoppers and stored at 5°C. They

were brought to room temperature before tests. The pH values were determined electrometrically.

Blood specimens were collected each hour for at least five consecutive hours. Urine samples were obtained every thirty minutes, as specimens ac-

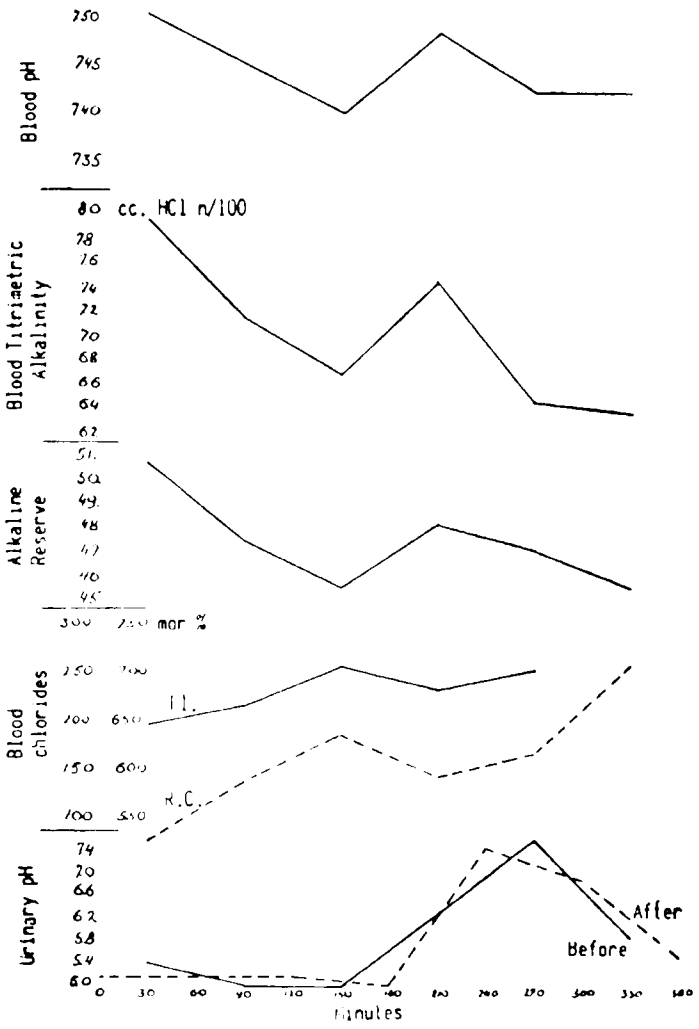

FIG. 204. In some cases the changes in several blood analyses parallel the changes of urinary pH. (Subject with metastatic melanoma)

cumulated in the bladder during the half hour preceding bleeding and again during the half hour following bleeding. The pH values of the urine specimens were plotted separately on graphs as A and B curves. Comparison was then made between the two urine curves and the curve representing the values of the blood titrimetric alkalinity, using time as abscissa. In another group of experiments using dogs, the bladder contents were drained at intervals of from five to ten minutes and blood specimens were obtained every thirty minutes.

Several preliminary tests were carried out to determine the degree of accuracy of the methods used. By employing a 0.001N NaOH solution, a probable error of no more than 0.1 cc. was found.

Comparisons between titrimetric alkalinity of hourly blood specimens and half-hourly urine specimens were made in thirty human and seven

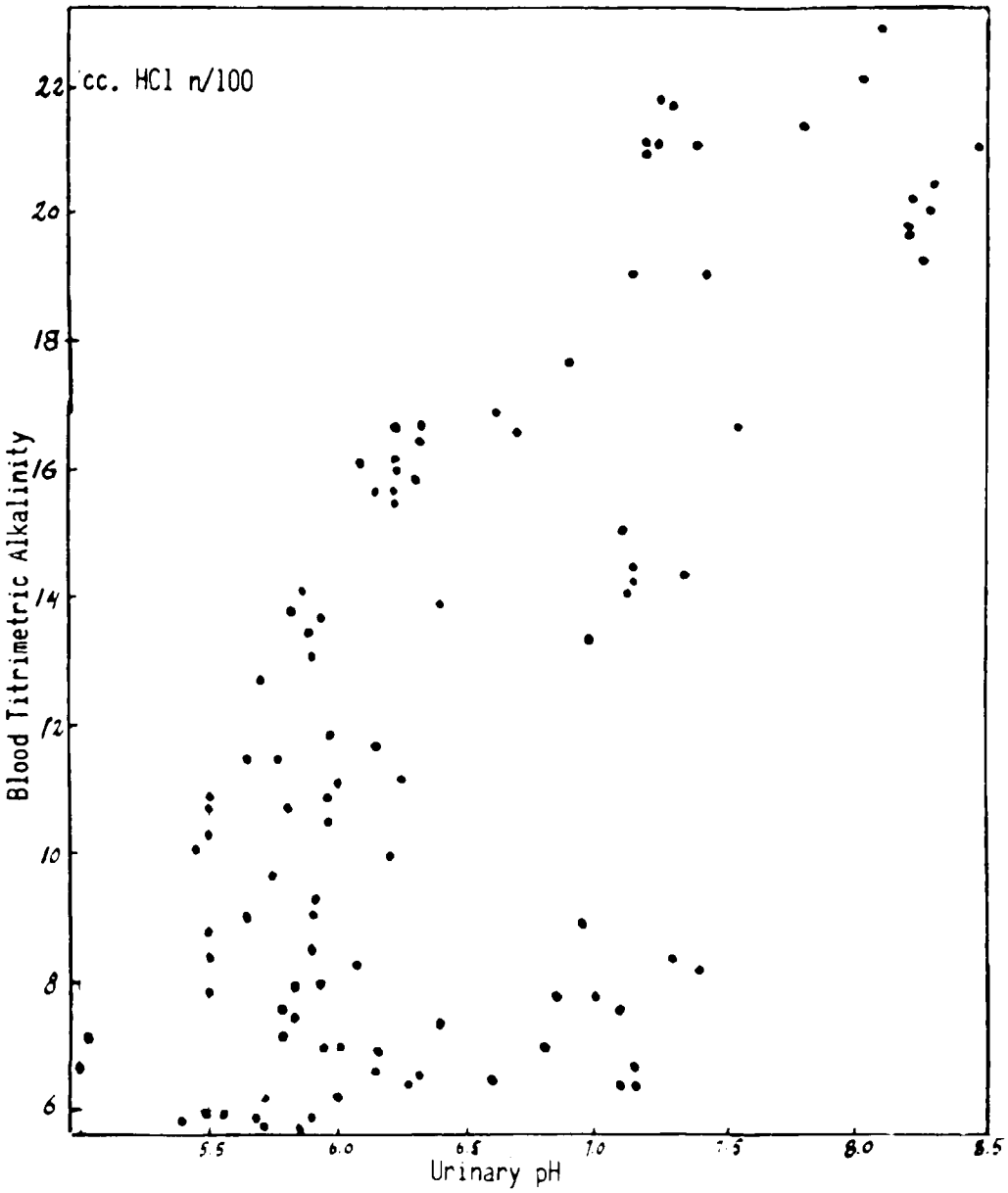

FIG. 205. The comparison between the concomitant values of urinary pH and of the titrimetric alkalinity of the blood shows that this relationship concerns more the occurring changes and less the absolute values of the findings. Urine samples with the same pH, in different subjects, are seen to correspond to blood samples with different titrimetric alkalinity.

canine subjects. In all cases, the curves of blood titrimetric alkalinity values showed a consistent parallelism to the pH curves of urine specimens that accumulated in the bladder during the thirty minutes preceding the bleeding and were collected at the time of venipuncture. *(Fig. 206)* The curves representing the pH values of urine specimens accumulated in the bladder dur-

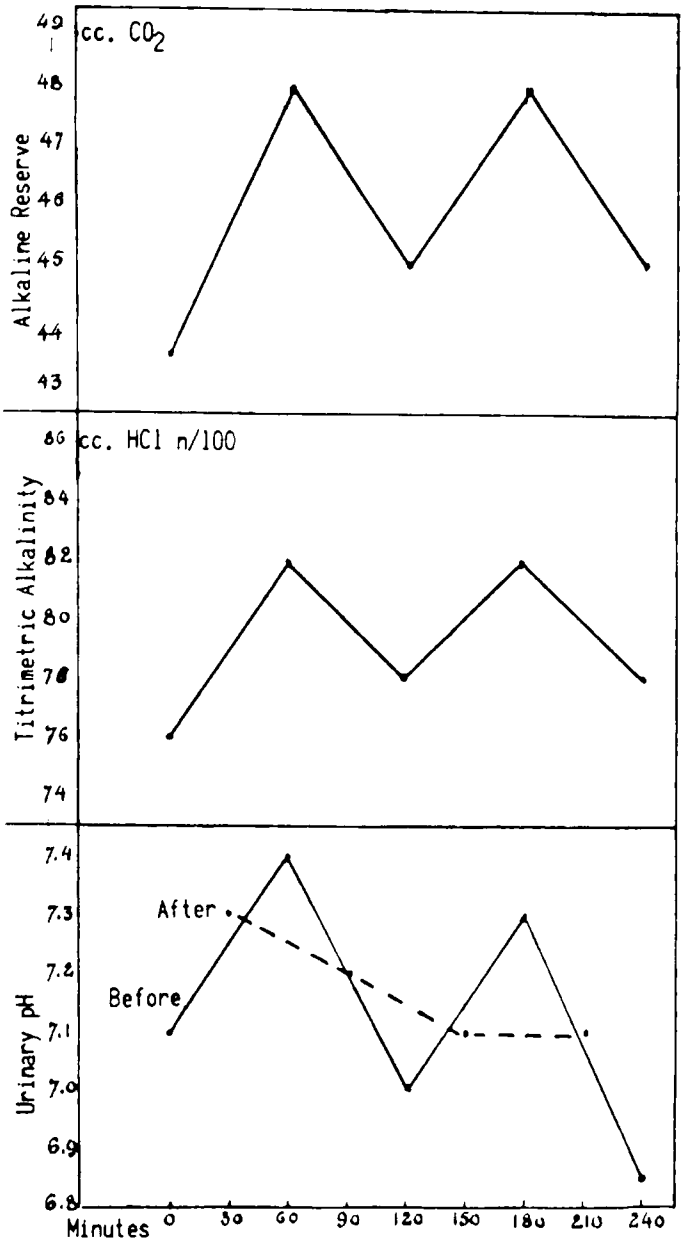

FIG. 206. Comparison of the hourly blood titratable alkalinity (in terms of cc. of .OIN HCl) and half hourly pH curves in a human subject. Urine curves A and B represent the before and after bleeding specimens. The parallelism between the titratable alkalinity of blood and the urine curve A is clearly shown. Curve B shows no correlation.

ing the thirty minutes after each bleeding (B curves) did not show the same consistent correlation to the blood titrimetric alkalinity. *(Fig. 207)*

In several tests, collections of half-hourly urine specimens were made fifteen minutes before and fifteen minutes after each bleeding. The same parallelism was found between the pH of urine accumulated in the bladder during the period from fifteen minutes before to fifteen minutes after bleeding, and the titrimetric alkalinity of blood specimens drawn in the middle

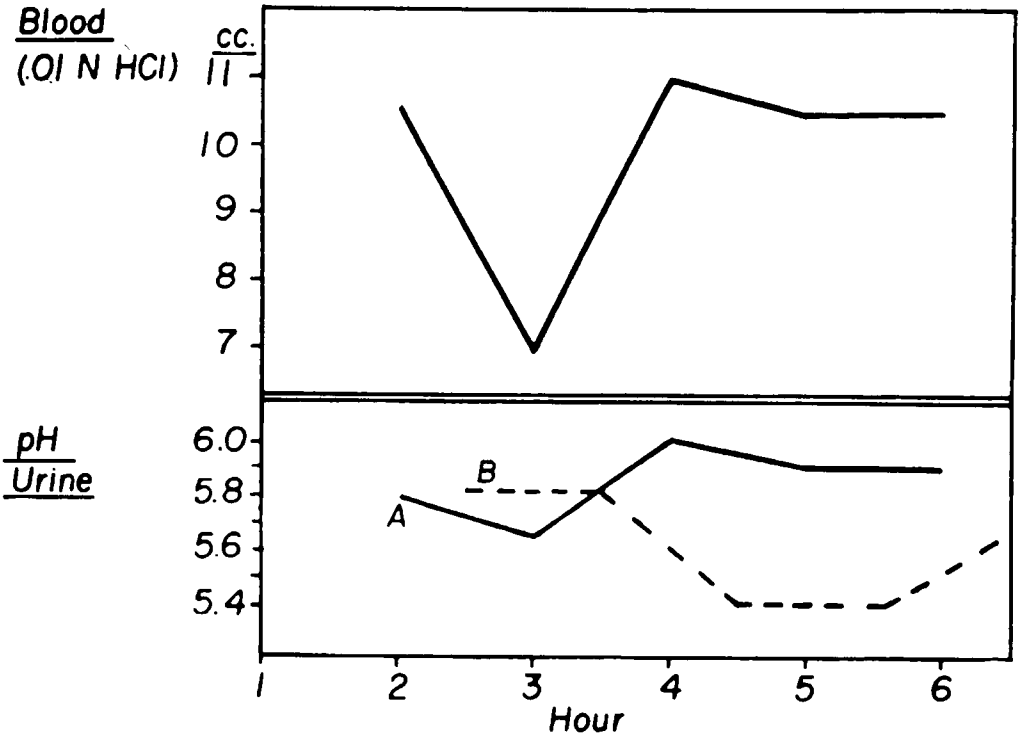

FIG. 207. Comparison of the curves representing hourly blood titratable alkalinity (in terms of cc. of .01N HCl) and urine pH in a human subject. Urine pH curve "Before" shows values of specimens accumulated in bladder during the half hour immediately preceding and collected at the time of bleeding, while urine pH curve "After" is for specimens collected one half hour after bleeding. There is a definite parallelism only between the curves of the titratable alkalinity of blood and the urine pH curve A.

of the urine collection period. The pH curves of urine specimens accumulated during the period from fifteen to forty-five minutes after each bleeding showed no consistent correlation.

When urine and blood specimens were obtained at shorter intervals, the same tendency of urine pH changes to precede the changes in blood titrimetric alkalinity was observed. In Figure 208 a rapid rise in urine pH is seen to begin within twenty minutes of the time of administration of sodium bicarbonate. The blood titrimetric alkalinity does not show an elevation for at least forty-five minutes.

These studies have shown that urinary pH variations correspond closely to changes in the values of an important factor reflecting acid-base balance changes of the blood, the titrimetric alkalinity. As a result, it has been possible to employ variations in urinary pH as indications of qualitative changes in acid-base balance of the blood for other studies.

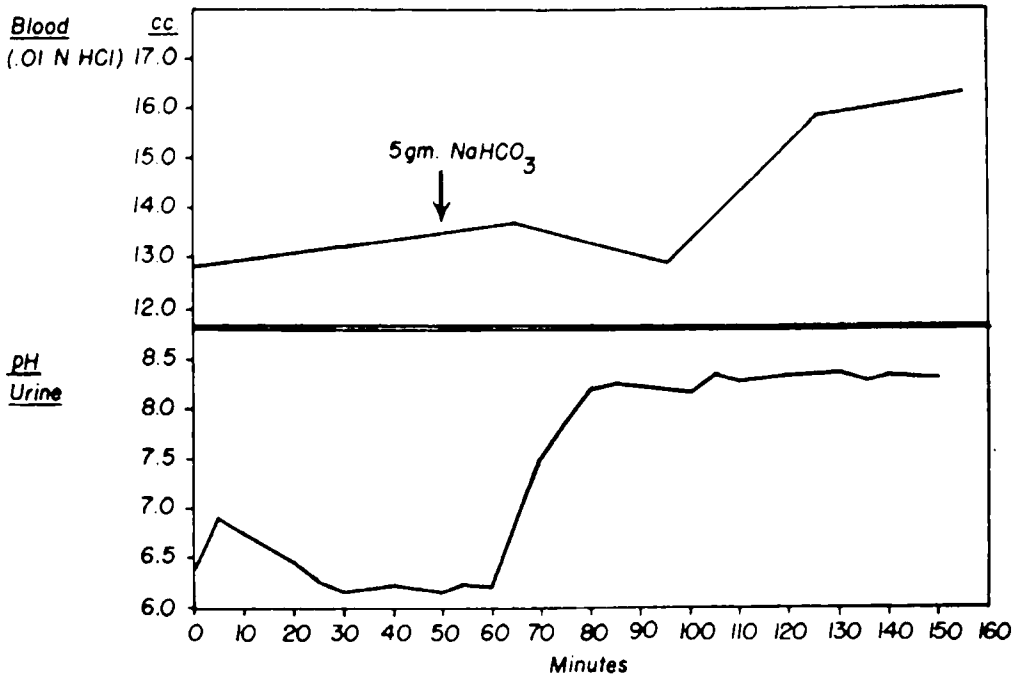

FIG. 208. The effect of the administration of 5 grams of sodium bicarbonate upon the *blood titratable alkalinity and urine pH curves* in dog. The elevation in urine pH is seen to precede by twenty-five minutes that of the titratable alkalinity of the blood.

Chapter 4, Note 3. Acid Pattern of Pain and Lactic Acid

We have investigated the relationship between pain of acid pattern and the appearance of lactic acid resulting from abnormal metabolism of carbohydrates. For this purpose we used the technique of Friedemann, Cotonio and Shaffer. In several patients it appeared possible to establish this relationship by measuring the lactic acid content of efferent blood from tumors during intensive pain of acid pattern. In a young man with a huge sarcoma of the knee, for whom the acid pattern of the pain had been established through its relationship to the changes in the urinary pH, such analyses could be carried out in the blood obtained from the big easily accessible efferent veins. At the moments of very severe pain, the amount of lactic acid had markedly increased. Values as high as 128 mg./100 cc. blood were found during these painful periods, contrasting with values around 30 mg./100 cc. blood in the period of calm.

Chapter 4, Note 4. Itching

Although we were especially concerned with pathological itching, we also became interested in physiological itching, especially in its relationship to sensorial sensations in general. Physiological itching can be regarded as a distinct sensation, not just a gradation of another sensation. Itching seems to have its own end organs. The propensity of certain regions of the body—such as the nasal mucous membranes, the skin near the nostrils, and the perioral and perianal skin—to itch in response to external stimuli can be correlated with the presence of such end organs. The proteopathic character of itching would also prompt us to consider less myelinized or even nonmyelinized nerves as its conductors. By analogy with pain, the existence of proper central centers could be conceived.

The most important characteristic of itching is that it can be induced by stimuli which, at other intensities, result in a different sensation such as touch, for instance. Although less manifest, other stimuli, such as heat and cold, also can induce itching. We have seen that stimuli which usually induce other sensations can produce physiological pain if they have an intensity above a threshold level. It is the intensity of the stimulus which determines whether it causes pain or a sensation of touch, heat or cold. Since pain appears if the stimulus is above the threshold level, it serves as a warning of a damaging incitation.

In studying itching under a similar aspect, it can be seen that it, too, is induced by nonspecific stimuli. But, for itching, the intensity of the stimuli is low. Everybody knows that an essential condition for the induction of itching is that the incitation be slight. This is easily seen for the skin, and especially the nasal mucous membrane, where a stronger stimulation will not induce itching but a touch sensation. Just as the intensity of a stimulus determines whether pain or touch is produced, so the intensity also determines whether itching or touch is felt. While the sensorial sensation of touch is induced by stimuli with intensities below those required for pain, itching is induced if intensities are below those required for touch.

The relationship of intensity of stimulus to itching, sensorial sensation of touch, and pain is shown in Figure 208 bis. This correlation explains why itching is present sometimes for a brief period when skin or mucous membrane sensorial sensation or even pain is induced. Immediately after an injury, for example, itching may be felt for a short time only to disappear just prior to the development of pain. The low intensity of the stimulus required explains a striking characteristic of itching: its disappearance when a stronger stimulus is applied. Thus scratching, which adds more intensive stimulation, makes itching disappear. The more violent the scratching, even to the point of inducing pain, the more effective it can be in eliminating itching.

The general reaction toward itching also appears related to the character of its induction. The individual responds to pain by fleeing or fighting in order to escape the intensive noxious incitation. As the incitation that produces itching is minimal by definition—the presence on the skin of a

minor irritant such as a fly or mosquito, for instance—scratching is sufficient to eliminate it. With a fly on his skin, the individual need not flee or fight, but only scratch. With the concept that itching can result from exactly the same type of stimuli as pain and touch, we integrate it in the group of sensorial sensations. We can then establish a separate sensorial subgroup for itching and pain. While other sensorial sensations inform us of the nature of the excitation—heat, cold, sound, taste, etc.—itching and pain inform us only about the intensity of the stimulus, not its nature.

Pathological itching, like pathological pain, is related to the existence of abnormalities. In addition to the differences in stimulus intensity required to induce itching and pain, their different nervous formations help explain their clinical separation. No patient we have studied has ever indicated any confusion as to whether his discomfort was due to severe itching or pain. The two sensations are seldom concomitant; usually they succeed one another. The fact that proteopathic pain and itching both seem to be conducted through unmyelinized nerves indicates why they can appear under similar conditions, as in nerve regeneration. This seems to have led to confusion between itching and pain. However, itching and pain observed during nerve regeneration can be clearly differentiated by the patient. The fact that the itching sensation is produced by stimuli of low intensity also explains why itching is so often present on skin or mucous membranes without appreciable pathology. Minimal changes appear sufficient to induce the sensation.

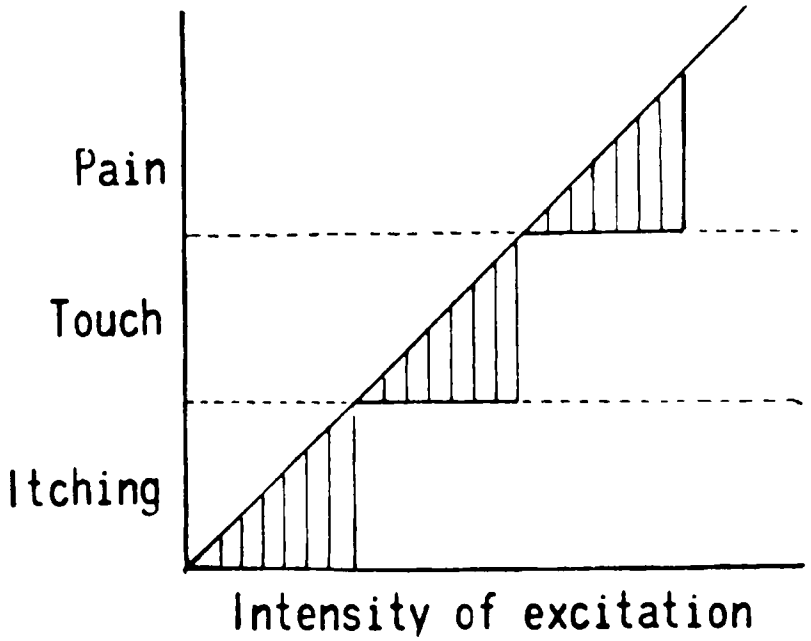

FIG. 208 bis. Similar to pain, *itching* represents a special kind of sensation, with the aim to inform about the intensity of the excitation. If this is very slight, it induces itching. If above the threshold, at which the incitation acquires a noxious character, it induces pain.

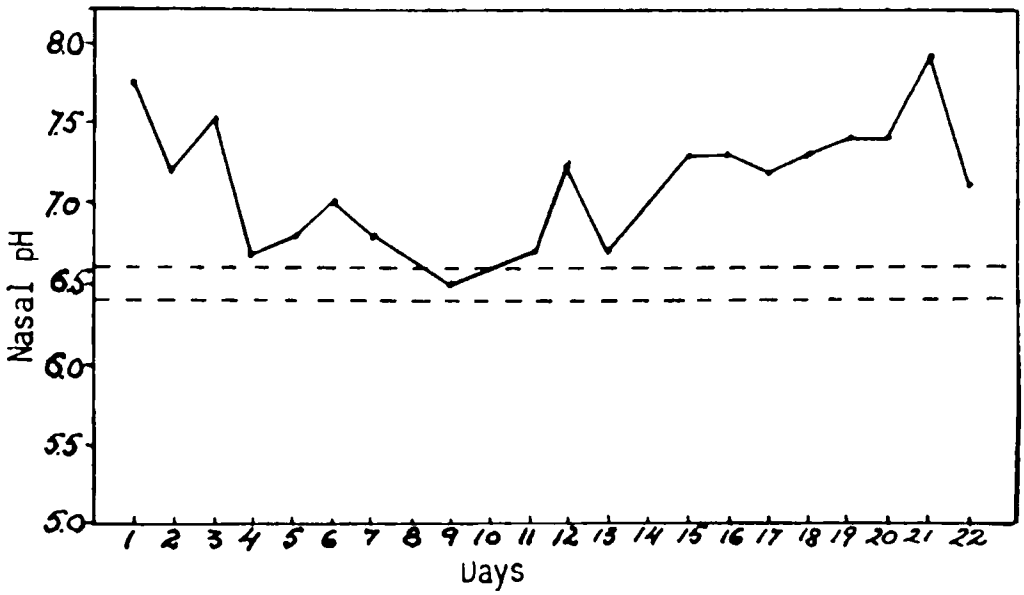

FIG. 209. *The nasal pH* measured with a glass electrode introduced deep in the nose shows the same dualism as the other analyses. With 6.5 as the average value, the curves of the nasal pH has more rapid and broader variations than other analyses. Curve of daily analyses shows values above the average line in a case of generalized melanoma.

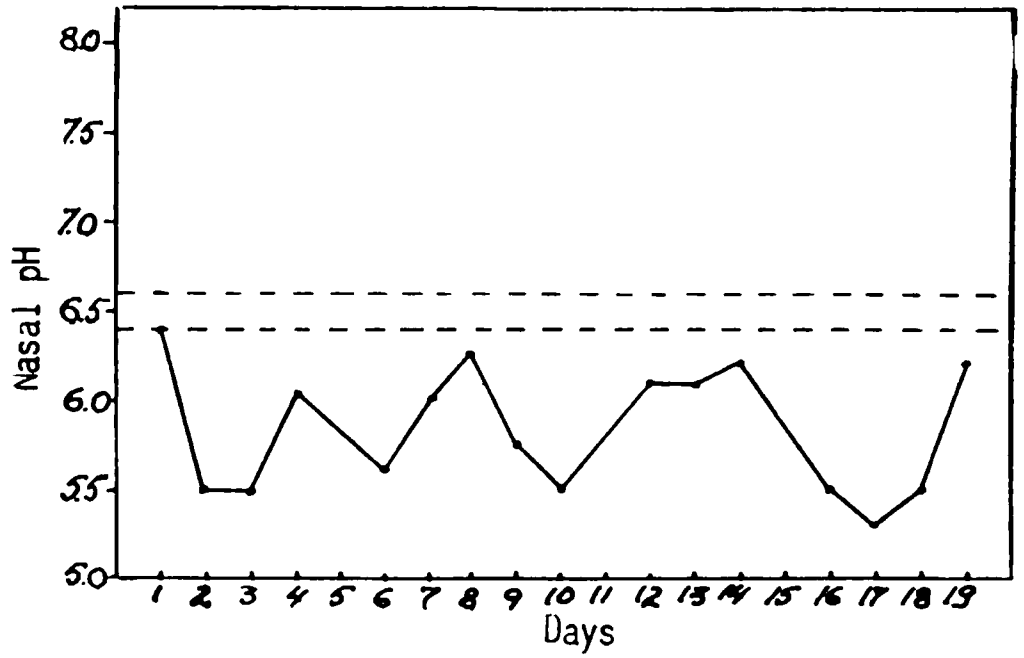

FIG. 210. The nasal pH shows persistent low values in a case of cancer of the liver.

Chapter 4, Note 5. **Nasal pH**

Nasal pH was measured using a portable Beckman pH meter and a glass electrode small enough to penetrate deep into the nose. In a research made with N. Buchanan it was found that valid data could be obtained only if the electrode touched the turbinate, otherwise marked differences in values were noted.

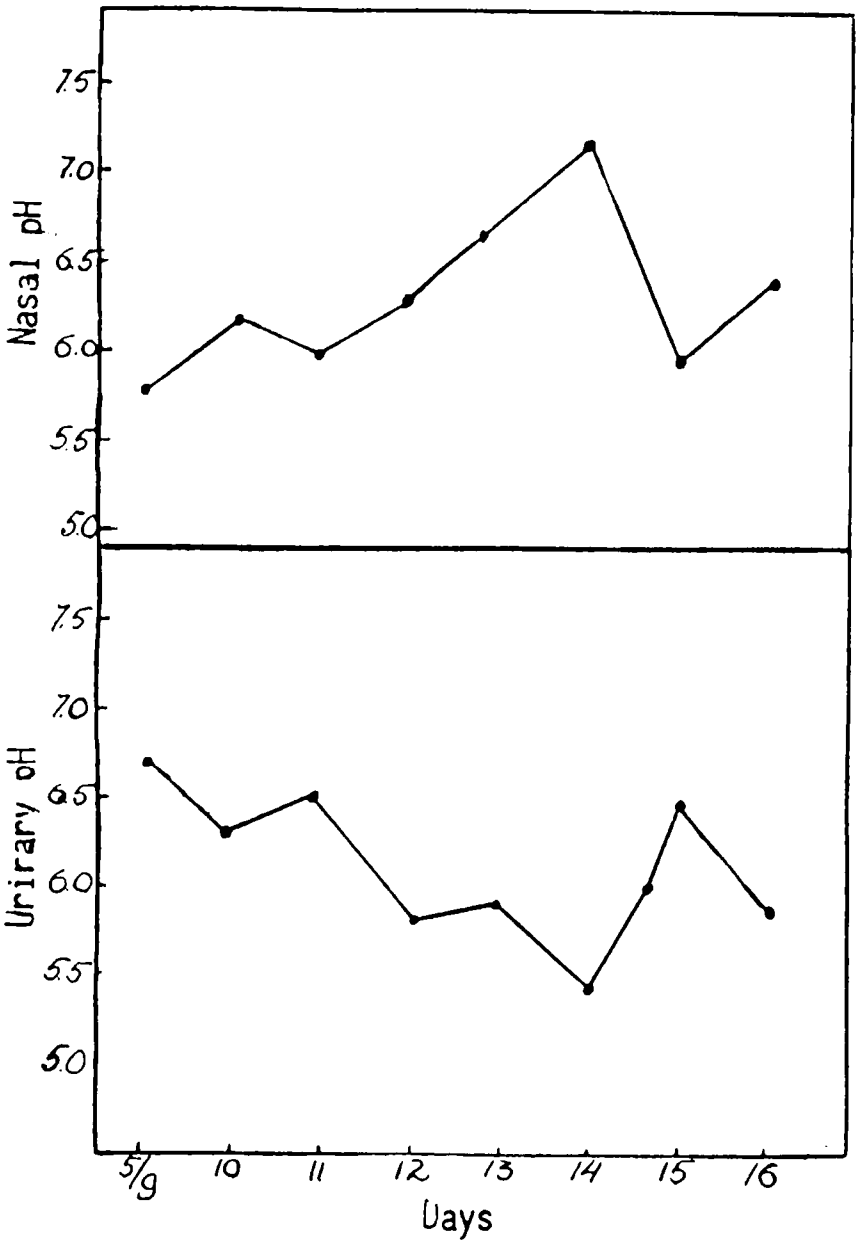

FIG. 211. The relationship between daily changes of the nasal and urinary pH shows opposite variations.

In a simplified method, cotton applicators were soaked in Guillaumin indicator solution with methyl red and bromothymol blue and left to dry. They were easily introduced to sufficient depth in the nose and left in place for at least two minutes. Color of the wet spots was checked with a colorimetric scale. Data obtained with glass electrode and colormetric applicator were found to coincide closely.

Two offbalances could be seen, one with the pH elevated sometimes even above 8. Figures 209 and 210 show curves of the offbalance in two patients. It is interesting to note that the changes in the nasal pH values parallel these seen at the level of lesions and are opposite to those concomitantly occurring in the urinary pH *(Fig. 211)* which parallel those of the titrimetric alkalinity of the blood.

Chapter 4, Note 6. **Wheal Resorption**

Interesting information could be obtained by analyzing the absorption of fluid injected intradermically in various subjects, and correlating the results with the existence of metabolic offbalances. We used the technique proposed by McClure and Aldrich, in which they measured the time needed for the disappearance of a wheal, resulting from an intracutaneous injection of a saline solution. A relatively extensive study of absorption was made in more than 500 subjects—both normal and abnormal. We present here a few of the conclusions from this study.

The average time necessary for resolution of the wheal obtained by the injection of .2 cc. of 7% NaCl solution, in normal subjects, was 23 minutes; the range was from 15 to 30 minutes. When deviations from these values were observed, they were consistent, in the sense that tests repeated at short intervals in the same general area in the same subject gave values in the same abnormal range. Abnormal values occurred in two directions. Resorption time was shortened in some cases and values as low as 1 to 2 minutes were noted. In the opppsite direction, values as high as 90 minutes were observed. These deviations from normal time could be related to local and general conditions. The presence of local or regional edema shortens the resorption time so much, that in some cases with massive edema, no wheal could even be realized. Shortening of time was found to be true for an edema, regardless of cause—inflammation, impaired local circulation as in phlebitis, impaired general circulation as in cardiacs or in renal failure. Lengthening of resorption time in cases of phlebitis provides valuable information on the evolution of the condition. The return of resorption time to normal values seems to indicate sufficient improvement to permit mobilization of the patient.

In subjects in whom no local factor could be considered to be responsible for changes in the resorption time of the wheal, we could see that abnormal variations had a direct relationship with the general offbalance present. In some subjects with a manifest offbalance of type D, wheal resorption time was shortened. Values as low as 4 to 5 minutes were obtained. Analysis of a number of cases indicated that this shortening of

resorption time meant a bad prognosis. A few patients with only values of 2 to 4 minutes died within a few days although other symptoms gave no indication of a fatal outcome within a short time.

Extended resorption time has been found in subjects with an offbalance of type A. Values as high as 60 to 90 minutes were found in subjects in whom all other analyses indicated this offbalance. It is also interesting to note the existence of slow resorption time for aged subjects. In a group of 80 patients ranging from 70 to 90 years of age, an average resorption time of 90 minutes was found. *(Fig. 68)*

Chapter 4, Note 7. **Eosinophiles**

The role of the blood as the secondary part of the entity organism has explained many of the peculiarities of its cells. Aside from the phagocytary functions that can be considered as a particular form of capturation, the leucocytes have to be recognized as acting as holocrine monocellular formations whose specific constituents are liberated by cellular lysis. We have seen that in the case of the neutrophilic granulocytes, the hydrolytic enzymes so liberated, strongly resemble the external secretion of the pancreas. Under this aspect, we have investigated the blood eosinophiles with a role similar to the Paneth cells of the duodenum.

The physiology of these leucocytes has to be sought in the acidophilic character of their granules. Morphological analysis of the eosinophile granule shows that it is formed by a content and a membrane, the last clearly seen in preparations in which the granules have lost their content. Like many other membranes, that of the eosinophile granule can be easily identified as being made partially at least by lipids being stained with dyes dissolving in lipids, such as black Sudan or Scharlach. However, it is the content of the granule with its ability to combine with acid dyes that indicates its specific characters. Under certain circumstances, when the blood is maintained in vitro between the slide and cover object for a certain time, the membrane and granule content are seen to separate. Before this occurrence, a lysis of the eosinophile leucocyte itself takes place. This is manifested through the breakdown of the cellular membrane with the lysis of the nucleus. It is in a second step that the eosinophile granule loses its content. Following it, besides the empty granules and lysed eosinophiles, characteristic Charcot-Leyden crystals appear. The correlation between these crystals and eosinophiles has been recognized and is generally accepted as occurring in vivo and in vitro.

Ayer (215) has shortened the process of lysis of the eosinophiles in vitro by treating the blood preparations with a detergent, aerosol. By repeating Ayer's experiments, the relationship between the appearance of the Charcot-Leyden crystals and the more complex process of lysis of the eosinophiles has become apparent. It could be seen that the crystals would appear at the site where the nuclei of the eosinophiles disappeared through lysis, and where careful observation of the granules reveals the loss of their eosinophilic content. The presence of empty granule membranes

stained by the fatty dyes, in addition to the lysed nuclei, would indicate the conditions under which Charcot-Leyden crystals appear. The eosinophilic content of the granule and products induced by the nuclear lysis represent the two factors that together result in these crystals.

Concerning the relationship between Charcot-Leyden crystals and eosinophiles, it is interesting to note the difference that exists between eosinophilic granules in various animals. Besides the morphological aspect which can be very different, apparently no Charcot-Leyden crystals are obtained from species other than humans and certain simians. This indicates that when the biological role of the eosinophiles is considered, we have to seek another common factor in addition to the morphological and chemical ones. It would seem that it is in their basic reactivity, *i.e.* in their capacity to bind substances of acid character, that the common character of all eosinophile granules has to be sought. This is also true for the duodenal cells.

Following this view, we initially tended to accord more importance to an antacid property than to any other, seemingly agreeing with other data obtained from this study. Among the substances found to be the principal constituents of these eosinophile granules, the alkaline amino acids, of which arginine is the the principal one, assume a very important role. According to the hypothesis we advance, these alkaline amino acids would represent the active factor of these granules and would be liberated by the eosinophiles when they disintegrate. The eosinophiles would intervene in physiology for the specific purpose of furnishing certain alkaline compounds in whose constitution the alkaline amino acids enter. The solubility of the granule content, when liberated, and the Charcot-Leyden crystals indicate, according to this view, that the main character of the eosinophile granule is its capacity to furnish alkaline compounds. Under special circumstances, they are able to act against substances with acid properties that result from the lysis of the nuclei, and together to form the Charcot-Leyden crystals.

The relationship of the disintegration of the eosinophile to the surface tension lowering agents is also interesting for the further liberation of the content of these granules. Just as for other granulocytes and lymphocytes, lysis is the characteristic fate of these cells and would constitute their most important character. As seen above, it can be related to the role of blood in the organization, *i.e.* as the secondary part of the organism level.

As for the other leucocytes, an important factor in the holocrinic role of the eosinophiles has been seen in the necessity of a maturation of these granules for their active intervention. When lysis was induced, it was seen to affect only the cells that had reached a certain degree of maturity, not only for the cells themselves but also for the granules. Young cells, recognized by more intense basophily of the cytoplasm, by lack of, or reduced lobulation of the nucleus, and especially by a neutrophilic or even basophilic character of the granules, do not break down. As in the circulating blood, immature elements are seen, the delay observed in inducing eosinopenia by various agents can be interpreted as corresponding to the time needed for the circulating eosinophiles to reach maturity, as an essen-

tial condition for their lysis. This situation was apparent in a particular case, that of eosinopenia induced through administration of the adrenal corticoids. Although this appears to have a direct effect upon the eosinophiles, a definite time, often even 24 hours, is seen essential in order to achieve the disappearance of the eosinophiles from the circulating blood. This delay has been related to the presence of eosinophiles, allegedly "resistant" to the corticoids. In fact, in studying the eosinophiles which persist after the administration of these hormones, we could see that they represent only immature elements, probably prematurely liberated in larger amounts from the bone marrow. The cells from which some will persist even for 24 hours after administration of corticoneoglucogenic hormones, do not show lysis in vitro nor the appearance of Charcot-Leyden crystals, and they present the tinctorial characters of immaturity for the cytoplasm and especially for the granules.

Granule maturation, which corresponds to the acidophilic character, seems to be the essential condition for the lytic intervention of these cells. In the physiological role of eosinophiles, an important aspect of maturation was seen in the relationship between the richness of these elements in the circulating blood and the processes in which a manifest local eosinophilia is induced, as through injection of parasite larvae or vegetal oils. A direct relationship between local and sanguine eosinophilia was apparent, the value of the former being the function of the latter. The ability of bone marrow to rapidly compensate the transitory eosinopenia following the passage of these cells into the tissues, has further directly connected the local richness in eosinophiles to the bone marrow's capacity to send new cells into the circulation. In all these changes, the prevailing factor has apparently been the degree of maturity of the eosinophile granulae, which seems to require a certain time to reach the desired degree which is the principal condition also for their physiological intervention.

Correlation between the biological intervention of the eosinophiles and the acidophilic character of the granules and their richness in alkaline amino acids has been confirmed in a study of the basophiles of the blood cells with granules having an opposite character. These granules have an acid content, as seen by their tinctorial affinity for alkaline dyes. They were also observed to contain heparine, a polysulfonated mucoid of frank acid character. The biological antagonism between heparine and alkaline proteins is well known. Protamines, the correspondent of histones for fish, are used to correct the excesses of heparin in the body, especially as therapeutic measures. Therefore, the antagonism between eosinophilic and basophilic granulocytes goes beyond their tinctorial characters.

Through the alkaline reactivity of eosinophiles as related to the fundamental separation of intervening constituents according to their positive or negative character, the antacid eosinophiles could be considered to be in the former group, while the basophiles, rich in lytic heparin, are in the latter.

We shall more fully discuss below the nature of the intervention of the eosinophiles after studying the role of a special group of constituents. For

the present, it seems that under abnormal circumstances, exaggeration in the amount of eosinophiles would indicate an existing predominance of conditions that correspond to agents of positive character, *i.e.*, with heterotropic tendency. The more precise antacid character of these cells further indicates the place that has to be reserved for the eosinophiles in the group of heterotropic agents. Under this aspect, the eosinophile would be seen as an agent of anti-acid character in the blood and tissues, conceived to act as a holocrinic cellular gland, *i.e.*, through the lysis of the corresponding cell. Therefore, the richness of the blood and tissues in eosinophiles would indicate a predominance of heterotropic tendency, while paucity in eosinophiles or their absence would indicate a homotropic trend.

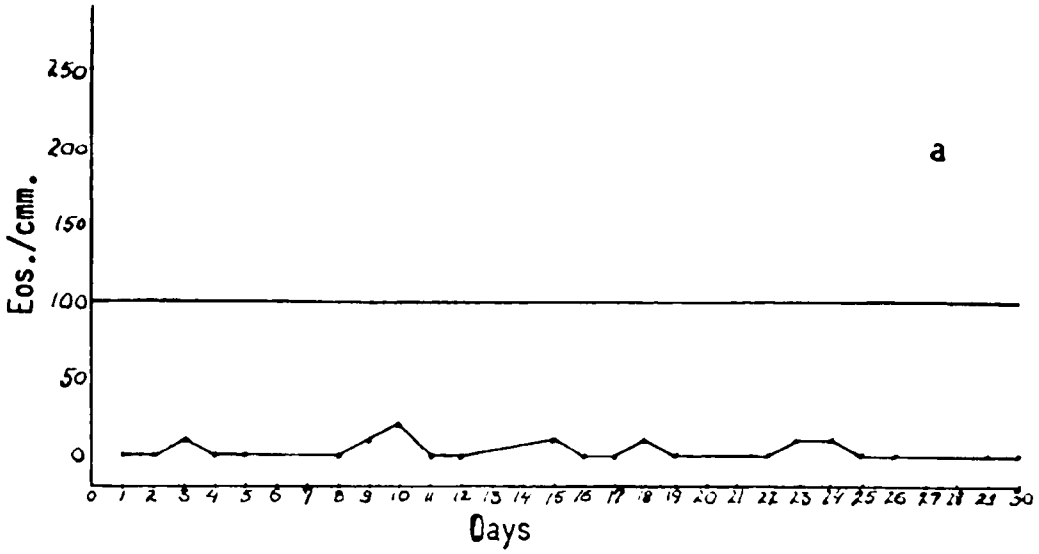

FIG. 212. Curve of blood eosinophiles in a case of breast adenocarcinoma with multiple metastases, showing values persistently below the average line of 100.

In order to understand this aspect of the eosinophiles, we tried to follow the changes in their amount in the blood in relation to normal and abnormal physiology. Study of the changes in the number of eosinophiles in the circulating blood under physiological conditions has indicated the existence of the same 24-hour oscillations as seen for many other constituents of the blood. A relationship is apparent between the periods corresponding to higher or lower quantities of circulating eosinophiles and the degree of activity of the individual. This appears to be opposite in humans who show diurnal activity, and mice and rats that show nocturnal activity. By experimentally changing the hours of light and dark for mice and rats, and through it the time of rest and of activity, the rhythm of change was reversed.

Following the concept of the intervention of eosinophiles in biological balance, we further investigated this aspect of the problem in relation to the dualism in abnormal conditions. Just as for other tests, we obtained an

average value in a large series of normal human subjects. Utilizing the Dungar technique for a direct count of eosinophiles, the value of 100 cells/1 cmm. was found to be the average value. An impressive direct correlation could be found between the amount of circulating eosinophiles and the two patterns of abnormality. In one group, that corresponding to type A, the number of eosinophiles appeared not only high but with their values fixed above the average value. High values were observed to persist for long periods of time. Figure 213 shows such a case. For the opposite pattern, corresponding to the fundamental type D, these values appeared to be below 100 and very often 0, persisting for a long period of time. *(Fig. 212)* In these dual patterns, the degree of abnormality could be related to the deviation in the number of these elements from the average value of 100 elements/1 cmm.

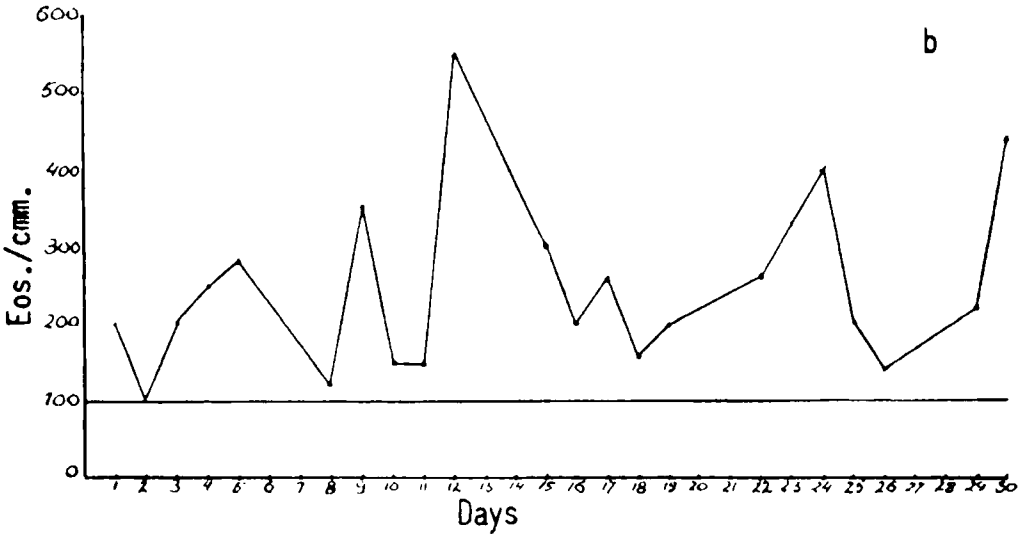

FIG. 213. Curve of the blood eosinophiles in a case of generalized melanoma showing values persistently above the average line of 100.

The relative facility with which the number of eosinophiles in the blood of an individual can be determined, has made it an important research tool for information regarding the balance between the two fundamental biological tendencies.

Chapter 4, Note 8. Total Blood Potassium

For a large-scale investigation—requiring as many as one hundred determinations a day—the technique of separating red cells from plasma appeared to be impractical. In view of the relatively minute amounts of potassium in plasma as compared to cells, we could utilize total blood instead of the cells. It was also found that by diluting the blood 1/10, the values obtained were in the same range as for serum potassium, a fact which permitted the use of the flame photometer without any change in the

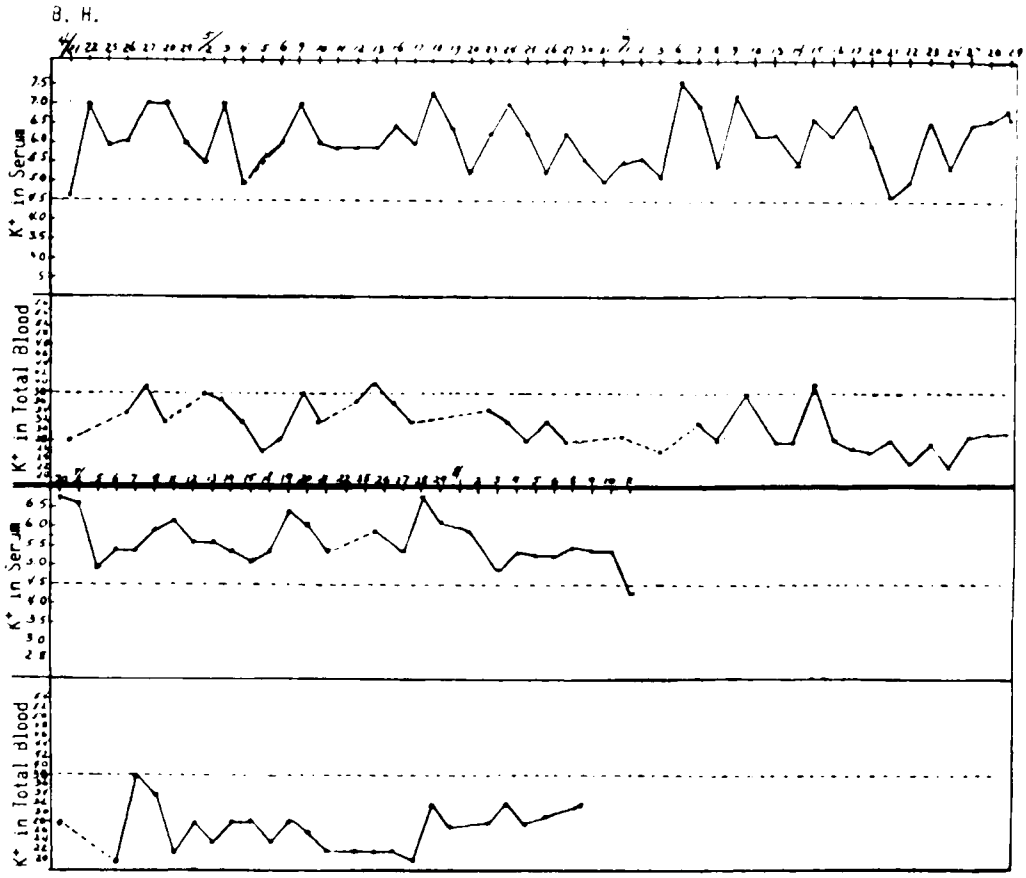

FIG. 214. Relationship between serum K+ and total blood K+ permits to recognize the nature of the changes concerning the intervention of this element. In a case of periarteritis nodosa, the high values of serum potassium and low values of the total blood potassium indicate an offbalance type D.

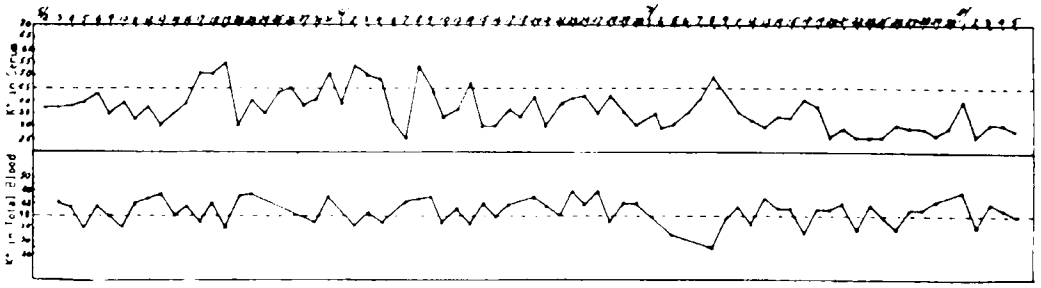

FIG. 215. Low values of serum potassium and high values of total blood potassium indicate an offbalance type A in a case of cancer of the gallbladder.

set-up of the apparatus. The blood was diluted with a 1% acetic acid solution in the pipette used for counting white cells. The pipette was shaken as for the count of cells, and the necessary amount taken from the diluted content. The potassium amount was determined and the result multiplied by 10. While the average value for the total blood was found to be around 38 mEq., values as low as 20 or as high as 60 were seen. *(Figs. 214, 215, and 216)*

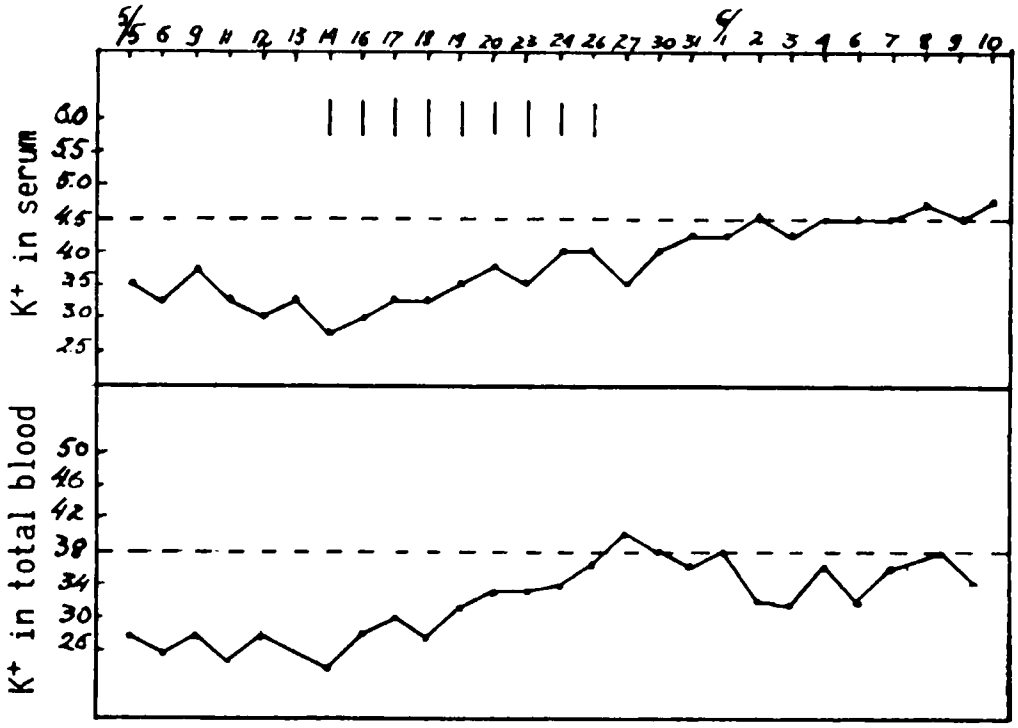

FIG. 216. Low values of potassium in serum and in total blood indicate a quantitative deficiency, in a subject with a liver adenocarcinoma. The administration of 40m Eq KCl daily, for 9 days brought the two curves to normal.

Chapter 4, Note 9. Sulfhydryl Determination

The catalytic effect of sulfhydryl groups on the oxidation of sodium azide by iodine was first described by F. Raschig (214), and F. Feigl (217) utilized it to develop the most sensitive qualitative test for the presence of sulfhydryl containing compounds. The reaction, initiated by mercaptans, sulfides, thiosulfates and thiocyanates, takes place as follows

$$2\ NaN_3 + I_2 = 2\ NaI + 3\ N_2$$

While this equation implies that the sulfhydryl compounds do not take part in the reaction, this is not entirely correct, because simultaneously, the sulfhydryl groups are oxidized by the free iodine. Accordingly, the reaction is carried out in a Warburg apparatus, where 1 ml. of 0.2 M sodium azide

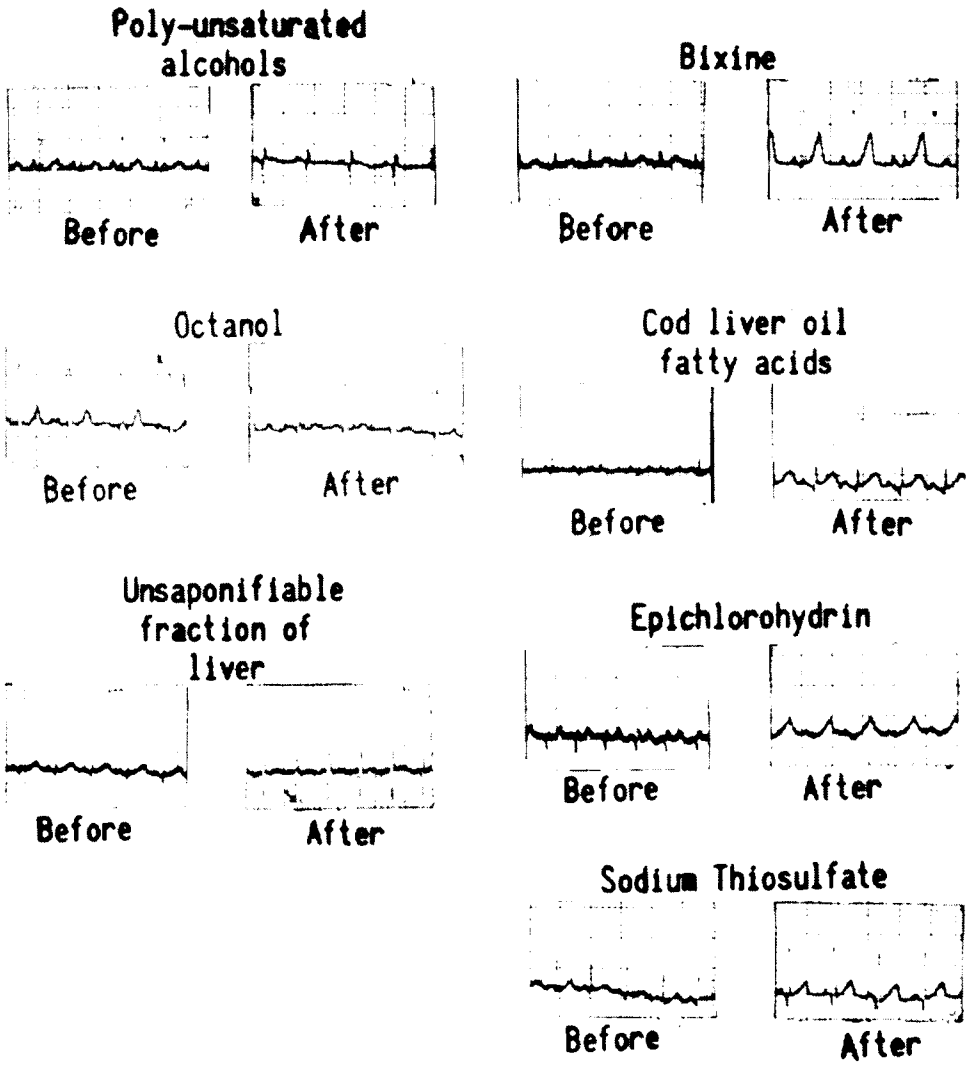

FIG. 216B. Electrocardiograms, in first lead in rabbits injected intraperitoneally with sublethal doses of different agents. In the group with lipoids with positive polar groups besides other changes, a flattening of the wave T is induced, which contrasts with a more elevated T for the group of lipoids with negative character.

and 1 ml. of 0.1 M iodine-potassium iodide solutions are mixed, while the sulfhydryl containing solution is kept in the sidearm, and then added to the reagents after temperature equilibrium is reached. Upon complete mixing, there is rapid nitrogen evolution, which, however, ceases within 13 minutes. The amount of nitrogen evolved is found to be linearly proportional to the content of the sulfhydryl groups, and on the average, 1 ml. of urine, 0.05 ml. of blood or 1 ml. of a 3×10^{-4} M sulfhydryl containing solution are amply sufficient for an assay. The method is thus well applicable to the determination of sulfhydryl levels, provided the method is standardized with the appropriate compound to be tested, as the catalytic effect of all mercaptans is not the same. (218)

Chapter 4, Note 10. Calcium in Urine

1 cc. of the urine was diluted in a test tube with 8 cc. of distilled water and the optical density of the mixture was determined. To the mixture, 1 cc. of a 1% solution of potassium oxalate and 3% of oxalic acid was added. After standing for 5 minutes, the tube was shaken and the optical density again was read. The difference, multiplied by 10, was divided by the two figures of the specific gravity of the sample. The value obtained was called the calcium index.

Chapter 4, Note 11. Urinary Surface Tension (ST)

The role of changes in the surface tension of various body fluids in normal and abnormal physiology has become of increasing interest. Some authors have gone so far as to consider the surface tension forces present at the interfaces separating entities, to be the most important factors in the boundary formations which serve to individualize these entities.

Considering multiple aspects of the problem, it appeared interesting to attempt, as a first step, to obtain information about the surface tension of different body fluids. It was as part of this program that urinary surface tension was investigated with the intention of utilizing the data to gain insight into changes related to the dualistic offbalances. Before we could proceed, it was necessary to resolve several problems, including the technical difficulties in measuring surface tension that result from the special constitution of the urine.

Technical Problems

Successive measurements of surface tension, when made on fluids formed by a single substance, consistently furnish the same value. But for fluids composed of two or more constituents, values vary from one moment to the next. This is explained by the fact that molecules of constituent substances have a tendency to migrate in the fluid, some accumulating at the surface, others concentrating in the bulk. (Gibbs dictum) The surface tension of different complex fluids has been found to vary according to the nature and amount of tensio-active substances present. And, study of the variations has furnished information about the nature of these substances.

In a fluid such as urine, containing many different substances, the problem of variations in surface tension is a major one. ST measurements, made without considering these variations, would be subject to serious errors. Examination of different samples of urine has shown great differences between values obtained at different times. Using Lecomte du Noüy's tensiometer (215) it could be seen that, for the same urine sample, values vary according to the length of time the sample is left to stand. Values progressively decrease as standing time increases. Similar changes are seen when the pendant drop method is used. (216)

Because of the fact that a certain time is needed for changes to take

place, the relationship between change and time was investigated. The study of various urine samples has emphasized the inequality which exists between them not only in the intensity of changes but also in the time necessary for the changes to take place. This fact has rendered useless the measurement of the surface tension of different samples if all are made at some given moment. Except for measurements made at frequent intervals, use of du Noüy's tensiometer has appeared to be inadequate for urine. Traube's stalagmometer also is unable to furnish values that take these changes into account.

Theoretically, it would appear possible to obtain measurements that would correspond to the surface tension for each drop at a desired moment by changing the rate of flow of the urine through the apparatus. But the differences between urines, related to changes in distribution of components, have made this inadequate.

With the pendant drop method, progressive changes which occur in the shape of the drop would appear to indicate the changes in surface tension. (216) Technically, it would appear necessary to obtain data as frequently as possible in order to follow changes which occur at various times. By using serial pictures, the changes, the moment of their occurrence, and their intensity can be studied accurately. Unfortunately, the complexity of the method, with the need for frequent pictures and involved calculations, prohibits its use for routine measurements and, consequently, for any broad clinical and experimental research.

It was under these circumstances that we returned to the capillary method which we considered capable of furnishing the desired data. Classically, the height of the ascending column in a calibrated capillary is used to calculate the surface tension. Height alone, however, is unsatisfactory, since it does not reveal the changes that take place. It was by studying the descent of the column in a capillary that we were able to obtain the data which we were seeking. We could show that the column does not descend with uniform velocity. It stops or slows down perceptively several times before it comes to rest at a fixed value. We could recognize that, for most urine samples, there is a first stop usually of several seconds duration. In some urines, this first stop is replaced by a marked slowdown in velocity of descent. The stop or slowdown is followed by renewed but slower descent and a second stop somewhat longer than the first. After another descent, often lasting more than 20 minutes, a new stop occurs.

The time of descent, the duration of the stops, and especially the heights of the column at which the stops occur, while reproducible for the same urine, vary widely with different samples. They would thus indicate different repartitions and the times when they occur. This technique of using the capillary consequently appears to be adequate for the study of the surface tension of complex solutions and particularly for the study of urine.

Each of the heights at which the descending column stops would indicate the surface tension for a particular stage in the repartition of the constituents. In studying this problem further, it appeared advisable to try to

have the capillary so calibrated as to permit a direct reading of surface tension values at these stops. The study of the relationship between the surface tension of a fluid and the height of the column has indicated the nature of intervening factors, their values, and under what conditions a direct reading is possible.

The fluid column remains stationary in a capillary tube when the surface forces which bind the column of fluid to the wall of the capillary are equal to the weight of the fluid column.

With σ representing the surface tension; r, the radius of the capillary tube; h, the height of the column; Δ, the specific gravity of the fluid; and g, the acceleration of gravity, we have $2 \pi r \sigma = \pi r^2 hg \Delta$. It can be seen that the specific gravity is the only factor related to the sample, other than the surface tension, which intervenes in determining the height of the fluid column.

According to this formula, the relationship between the surface tension and the specific gravity of the specimen is: $\sigma = \frac{\Delta rhg}{2}$. The same height of the column is obtained if the relationship between surface tension σ and σ' of two different liquids with the specific gravities Δ and Δ' fulfills the condition: $\sigma = \frac{\sigma' \Delta'}{\Delta}$.

If measurements with a capillary tube having a bore radius of 0.5 mm. are made in New York City, where the acceleration of gravity is 981 upon water which at 18°C has a surface tension 73 dynes/cm., the height of the fluid column is found to be 6.0 cm. and the relationship between σ and Δ, expressed in the cgs. system is $\sigma = 73 \Delta$.

A capillary tube thus can be calibrated to permit the direct reading of the surface tension in dynes/cm. for any liquid having the same specific gravity. For fluids of different specific gravity, the same capillary tube can be used if a correction of 0.073 dynes/cm., is made for each 0.001 increment of the specific gravity.

Urinary specific gravity values encountered clinically range between 1.001 and 1.035, with an average value around 1.015. Tubes calibrated to measure urine specimens with specific gravity values at either extreme can yield errors in the surface tension of as much as 2 dynes/cm. In order to minimize the degree of error for routine laboratory use, the capillary tube has been calibrated to correspond to a fluid with a specific gravity of 1.015. The maximum error of the surface tension values for the extremes of specific gravity clinically observed will be reduced to approximately $\pm$ 1 dyne/cm. in this way. Furthermore, the fact that sodium chloride concentration is one of the important factors inducing different values for urinary specific gravity reduces the influence exercised by specific gravity upon the height of the column. Sodium chloride represents a negative surface active substance. It will raise the surface tension values as its concentration increases because of its tendency to migrate from the surface toward the bulk of the fluid. This will partially decrease the influence exerted by the specific gravity of the urine. Since the surface tension values of human urine speci-

mens measured by this method have been found to vary between 73 and 50 dynes/cm., the error for extreme values of specific gravity is less than 5% and is not clinically significant. If more precise values are desired, the necessary correction can be made by adding or subtracting 0.073 dynes/cm. for every .001 difference in the sample above or below the specific gravity for which the tube is calibrated (*i.e.* 1.015).

The temperature of the urine to be tested is another factor which intervenes. Although the fluid rapidly attains the same temperature as the capillary walls, it is advisable to perform measurements when the temperature of the fluid is around 18°C, since the surface tension of a liquid decreases as its temperature increases. For clinical use, corrections for differences in temperature are not considered necessary.

Design and Calibration of the Urotensiometer

In order to obtain direct readings of the urinary surface tension values with a maximum error of ± 1 dyne/cm., the previously discussed factors were taken into consideration in designing and calibrating the urotensiometer which we conceived. *(Fig. 217)* The glass capillary tube has a bore

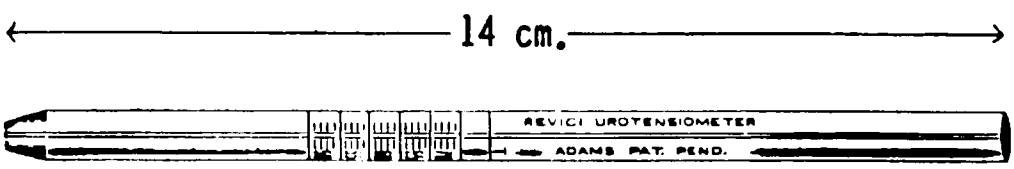

FIG. 217. Urotensiometer—calibrated to indicate by direct readings in dyne/cm the surface tension of fluids having a specific gravity of 1.015.

diameter of 0.5 mm. and is approximately 14 cm. in length. It is calibrated to indicate the surface tension of a fluid with a specific gravity of 1.015 directly in terms of dynes/cm. in the following manner: a continuous column of distilled water at 18°C is drawn up to about three-fourths of the height of the tube and allowed to descend with the tube maintained vertically. The point at which the top of the column stops is marked. It represents a surface tension of 73 dynes/cm. for water having a specific gravity of 1.000. In order to make the necessary correction for a fluid having a specific gravity of 1.015, the distance between this point and the tip of the tube is divided into 74 (instead of 73) equal parts. The tube is calibrated down to 50 dynes/cm. since lower values have not been encountered. In the tubes manufactured by Clay Adams, New York, the markings are permanent. The split line feature of the scale permits easy visualization of the meniscus. The encircling lines help in maintaining the tube in the vertical position.

The Measurement of Urinary Surface Tension with the Urotensiometer

To determine surface tension by means of the Urotensiometer, the tapered end of the tube is introduced into the bulk of the urine specimen.

The fluid is drawn slightly above the highest mark by mouth suction and evacuated several times by positive pressure. The tube is again filled to the same point, care being taken this time that no air bubbles interrupt the continuity of the fluid column. The tube is removed from between the lips, and the tip of the capillary is then gradually raised toward the surface of the fluid. When the top of the column descends to the top line (T) of the scale, the tip of the tube is removed from the fluid and maintained in a vertical position at eye level. The descent of the top of the column can best be observed by viewing the meniscus between the ends of the split line calibration markings. The top of the column descends within one or two seconds to an initial point (P_1) where it comes to a temporary halt or its rate of descent suddenly slows perceptibly. The column again slowly descends, coming to rest after several minutes at a second point (P_2). After some time, the descent may again be resumed at a much slower rate until a third and final stopping point (P_3) is reached after more than fifteen minutes. For routine measurements, the first reading (P_1) is considered as the surface tension value of the urine. This corresponds roughly to the surface tension value of the specimen before any important secondary redistribution of molecules has taken place.

The capillary tube should be thoroughly cleaned with distilled water after use. It is well to check the tube before each series of measurements, using distilled water at room temperature. If the check readings are above 74 or below 73 on the scale, the tube must be carefully flushed through with distilled water by means of a suction pump. Occasionally, water alone may not be sufficient and it will be necessary to clean the tube with sulfuricochromic cleaning solution, followed by thorough flushing with water, in order to obtain correct check readings. When the tube is not in use, it is best left standing in a glass beaker containing distilled water.

Surface Tension in Clinical and Experimental Research

The Urotensiometer for the first time makes possible determinations of the surface tension of urine and other physiological solutions as a routine laboratory procedure. The highest surface tension value for urine encountered clinically is 73 dynes/cm., and this is correlated with a minimal quantity of surface-active substances. The lower the surface tension of the urine in dynes/cm., the greater the amount of tensio-active agents present in the specimen. A surface tension of 52 dynes/cm. is the lowest clinical value that we have found by this method in more than 100,000 measurements made during the last 12 years.

The first problem concerning the meaning of the different values of urinary surface tension arose when it was observed that usually the urines with low specific gravity have high surface tension, while those with high specific gravity have low surface tension. The direct correlation between the values of surface tension and specific gravity of the samples thus had to be investigated with the supposition that the amount of water in the urine will have a great influence, by itself, on surface tension. While a correlation between surface tension and water content is often observed,

it is not a cause and effect one. Urinary samples with a specific gravity as low as 1.003 were seen with a surface tension of 58 dynes/cm. while samples with a specific gravity as high as 1.030 had a surface tension of 70. Although very seldom encountered, these values have invalidated the supposition that it is the amount of water in the urine which determines the value of the surface tension, so that from the analytical point of view determination cannot be substituted.

The Nature of the Intervening Substances

The existence of several values for the surface tension of urine has suggested the intervention of different substances in the determination of surface tension. We used the study of the changes induced in the three values of P obtained for a sample. Different repartition capacities were considered as corresponding to different groups of substances. Several methods were used in order to identify these substances. In one method, different constituents of the urine were separated by using solvents or absorbents, or by allowing the constituents to assemble at the surface.

The fact that the solvents, if they remain in the fluid even in very minute amounts, influence the surface tension, has largely handicapped their use. However, when lipid solvents were used and could be thoroughly eliminated, the treated urine showed a change in surface tension, especially in P_1 values. With the use of activated animal charcoal absorption, all the P values were changed toward higher values.

M. Bier in our laboratories has studied the nature of the surface-active constituents, separating them from urine by using the fact that they assemble at the surface. Urine was made to foam by passing an inert gas through it. The foam—and, with it, a high proportion of surface-active substances was separated. By repeating the procedure, the separation could be pushed far enough so that it could be seen that the ST values, especially those of P_3, were influenced. Analyses of the fractions obtained indicated that lipids would intervene in determining the surface tension revealed by the P_1 value, while proteins would intervene for the P_3, *i.e.*, after a repartition requiring a specific time. We have tried to confirm these preliminary data by adding the agents to urine and following the changes induced.

The addition of minimal amounts of soaps to urine has been found to induce a change in all P values and especially in P_1. The addition of billiar salts changed P_2 values, while the addition of proteins, such as albumin, influenced the values of P_3. It would appear from this preliminary research that while P_1 changes are related to an increase in fatty acid derivatives, P_2 changes are related more to the intervention of billiar acids, while proteins and amino acids exert greater influence on the values of P_3.

This explains why surface tension, corresponding to P_1, is still high in urines rich in albumin, and sometimes also in those with billiar acids. It would also explain the observation in the Hay's Test with sulfur flower in urine, that the sulfur starts to fall quickly if the urine is left standing for a while, but for the same urine this fall occurs only after a certain time if the sulfur is added to urine immediately after stirring. With surface tension

affected by fatty acids even in minimal amounts, ST changes in relationship to conditions where these substances intervene are particularly interesting. It is chiefly with these data in mind that we tried to investigate surface tension in relation to normal and abnormal physiology.

Surface Tension and Normal and Abnormal Physiology

The ability to measure surface tension rapidly and accurately enough, even for very small amounts of fluid, has made it a preferred method for many investigations. In addition to clinical applications, where the information furnished has been especially valuable, we have utilized this method in experiments in animals.

Time of the Day and Urinary Surface Tension

Urinary surface tension measurements were made in several normal subjects at hourly intervals. In order to eliminate the influence exerted by exercise and food, the subjects were kept in bed for a few hours preceding

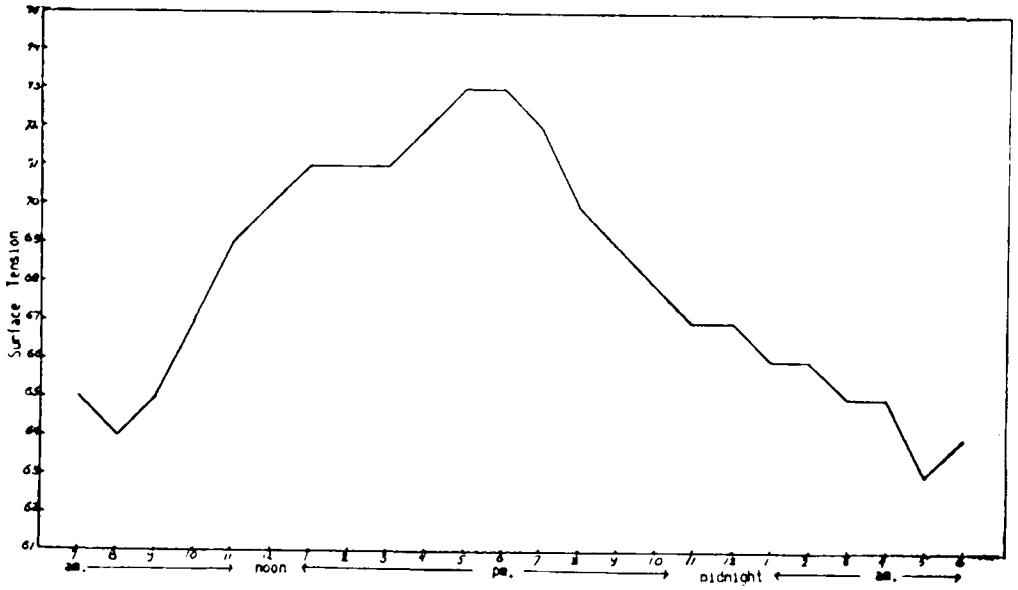

FIG. 218. 24-hours hourly urinary surface tension value of a 30-year old normal male, kept resting and with a constant hourly food intake, showing a maximum in the afternoon and a minimum around 5 a.m.

measurements. During this period, and the entire period of the experiment, the subjects were permitted to leave their beds each hour to void. Throughout the experiment, they were given the same kind and amount of food each hour. This eliminated as variable the influence of food and activity. Figures 218, 219 and 220 and Table XXVI show samples of the curves of surface tension in such cases. A 24-hour diphasic curve can be noted.

Surface tension in mice under similar conditions, however, shows differences. A group of 20 mice kept in cages were used. By slight squeezing of

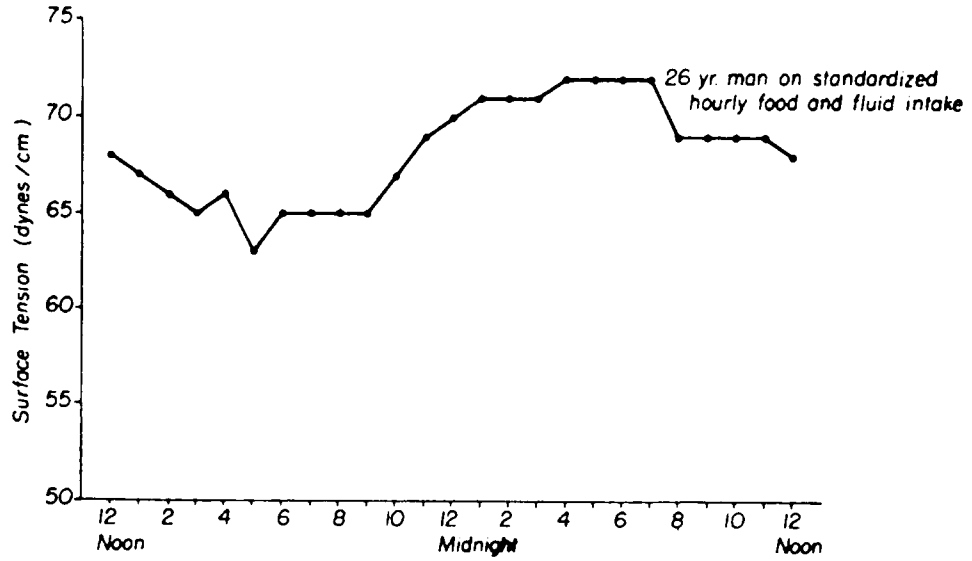

FIG. 219. Curve of the urinary surface tension in a 26-year old male on standardized hourly food and fluid intake showing a maximum toward the early morning hours and a minimum toward the evening.

the lower abdomen, a few drops of urine were obtained in a little cup and used for surface tension measurement. Changes seen in Figure 221 show that with the passage of time, there is a dampening effect on the curve. This has made us doubt that the intervention of stress in these cases can be responsible for the changes. In order to eliminate stress as a factor, a second group of experiments was done in which urinary samples were obtained

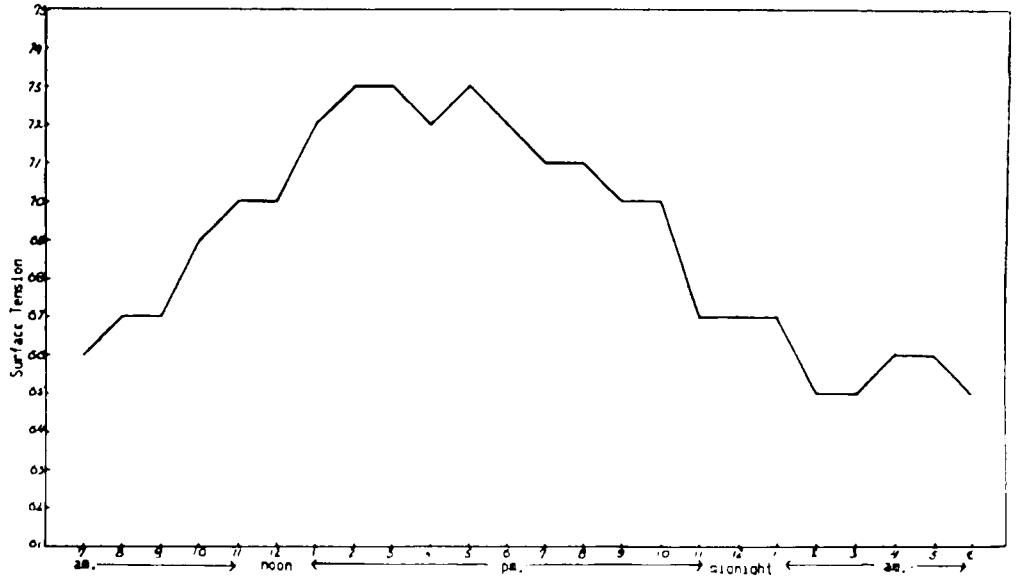

FIG. 220. Curve of the urinary surface tension in a 27-year old female on standard hourly feeding, with a maximum in the afternoon and a minimum in the morning.

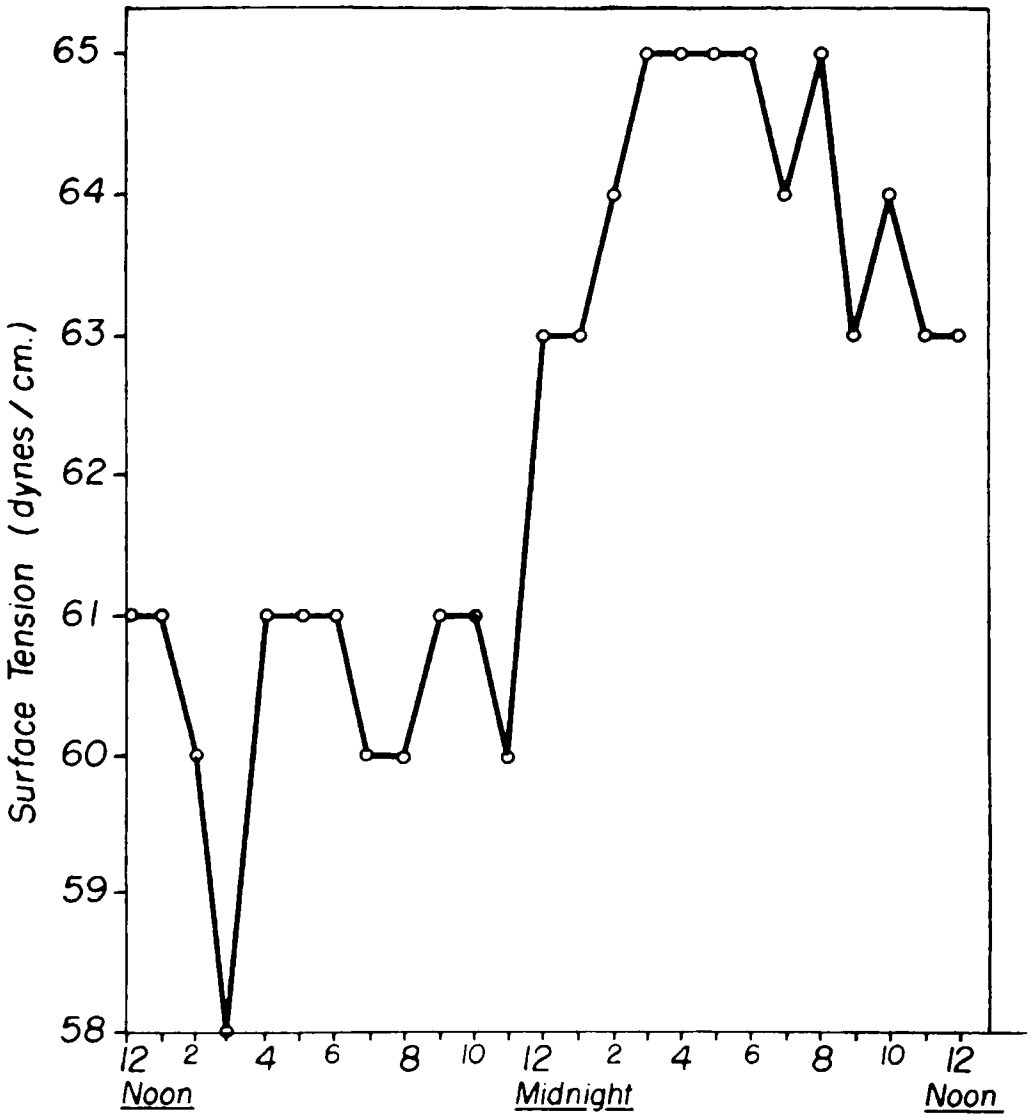

FIG. 221. Average value of surface tension in the urine of 20 mice, obtained every hour, showing variations with a dampening character.

each hour from a different animal. Thus, each animal provided a sample of urine only once and was not under stress. Under these conditions, the dampening effect was not present. A curve showing only two phases in 24 hours was obtained. The ST curves for humans and mice are opposite. During the period when high values occur in humans, low values occur in mice, and vice-versa. Because such opposite variations between humans and mice were found for many other analytical data and were considered related to the nocturnal activity of mice, a third group of experiments was performed in which the mice were kept in darkness during the day and under light during the night in an attempt to change the rhythm of their activity. After three weeks, there were no marked changes in analytical

data obtained in these mice. It is possible, however, that more time is required to induce changes in surface tension by altering the rhythm of mouse activity. *(Fig. 222)*

TABLE XXVI
SURFACE TENSION IN NORMAL HEALTHY 32 YEAR OLD MAN AND 27 YEAR OLD FEMALE ON STANDARD HOURLY FEEDING

Hour	Male S.T. in Dynes/cm.	Female S.T. in Dynes/cm.
7 a.m.	65	66
8 a.m.	64	67
9 a.m.	65	67
10 a.m.	67	69
11 a.m.	69	70
12 Noon	70	70
1 p.m.	71	72
2 p.m.	71	73
3 p.m.	71	73
4 p.m.	72	72
5 p.m.	73	73
6 p.m.	73	72
7 p.m.	72	71
8 p.m.	70	71
9 p.m.	69	70
10 p.m.	68	70
11 p.m.	67	67
12 Midnight	67	67
1 a.m.	66	67
2 a.m.	66	65
3 a.m.	65	65
4 a.m.	65	66
5 a.m.	63	66
6 a.m.	64	65

Surface Tension in Normal Humans and Animals

From the first analyses of urinary surface tension in groups of individuals it could be seen that certain changes common for all were taking place. There were days when all subjects had higher relative values and other days when lower values prevailed. Since there was no common dietetic or habit factor for all the subjects studied, we searched for environmental changes that might be the immediate cause of these variations. In collaboration with P. Teitelbaum we made the following experiment, using 80 rats divided into four groups. One group consisted of females of the Wistar strain and a second consisted of males of the same strain. The remaining two groups were composed of 20 females and 20 males of a black hooded strain. The animals were maintained in groups of five in separate cages on Purina and water ad lib. They were kept in a nonconditioned room. The experiment was conducted for one month, from May to June.

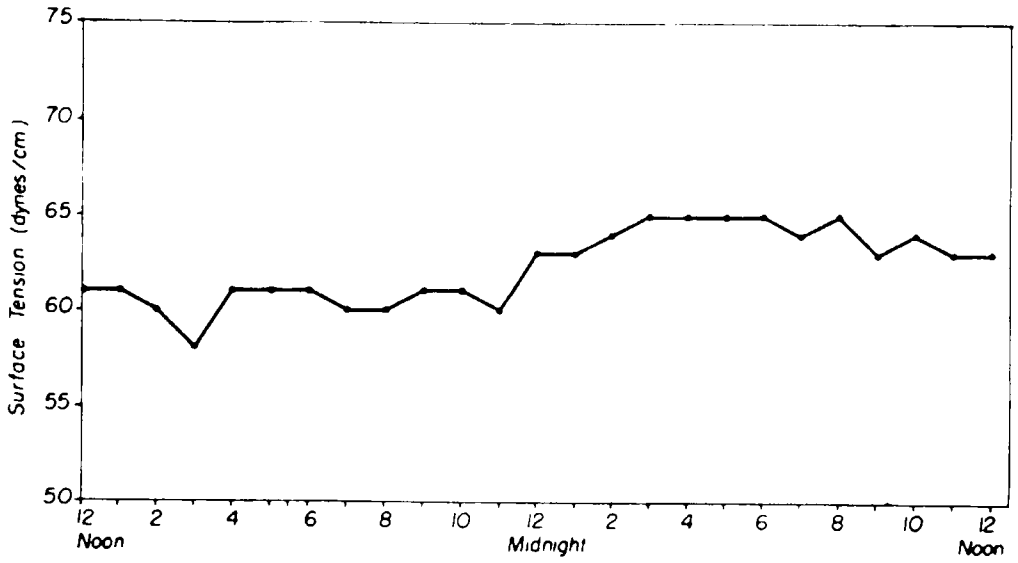

FIG. 222. Average hourly values in the urinary surface tension of groups of 5 mice, the group being changed each hour.

Urine was collected in a small vessel by keeping the animal firm and pinching the lower abdominal skin. Surface tension was measured a few minutes later. In each group, animals which did not give urine under this procedure for several consecutive days were replaced by others.

Samples of urine were obtained 6 days a week, between 9 and 10:30 in the morning. From the data obtained, an average value was calculated for each group, and the respective values were plotted in curves having the days as abscissae. The values for the female group were higher than for males. No differences were seen between the two strains. And all four curves showed the same variations at the same times. Thus it appeared clear that the variations were related to some external factor acting upon all the animals. We compared the ST curves with others traced for different environmental values present at the time of observation. Such values—barometric pressure, electrostatic value and temperature—were obtained from the Weather Bureau and the curves for the area at the hour of the experiment traced. Of them all, only the curve for temperature change was significant. The ST was seen to rise each time that the temperature fell and fall when the temperature rose. *(Fig. 223)*

This correlation was further studied by using induced rather than natural temperature changes in the following experiment which was made in collaboration with E. F. Taskier.

Adult female CF_1 strain mice were divided into three groups of 20 mice each. They had ad lib access to food and water. One group was placed in an incubator in which the temperature was maintained at 37°C. A second group was kept in a refrigerator at 8°C. The third group served for control and was maintained at ordinary laboratory temperature which ranged between 20-25°C.

Because of the diurnal pattern of surface tension variations, urine specimens were collected at the same hour every day. During a period of 22 days, daily urine specimens were obtained between 9 and 11 A.M. This was easily accomplished by firmly gripping a mouse in one hand by the scruff of the neck and tail. With the finger of the other hand, the lower

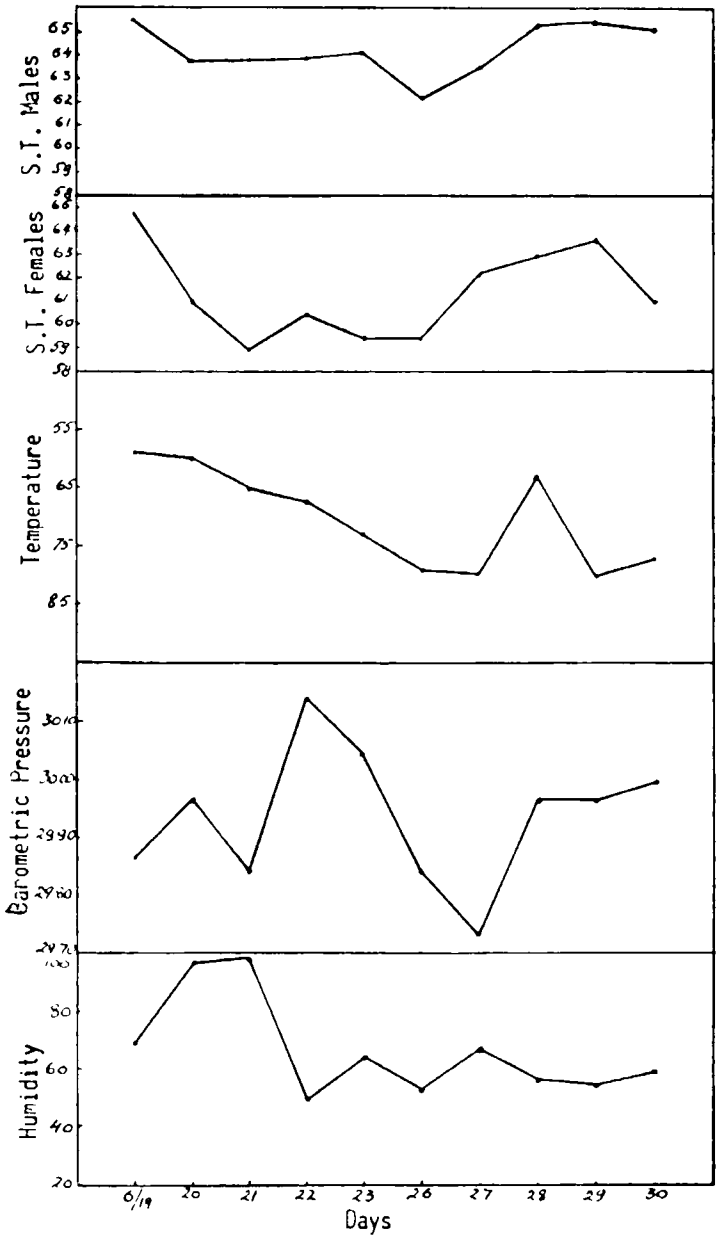

FIG. 223. Comparison between weather data and the average value of the surface tension in 40 male rats and 40 female rats. It shows a relative parallelism with the curve of the barometric pressure and a more consistent relationship with the inverse curve of the temperature.

abdomen was gently massaged, causing the animal to void 2-5 drops of urine into a small glass cup.

The surface tension of each specimen was determined within a few minutes after it was obtained.

The values obtained from day to day were charted for each individual mouse; the average for each group maintained under different temperature conditions also was determined.

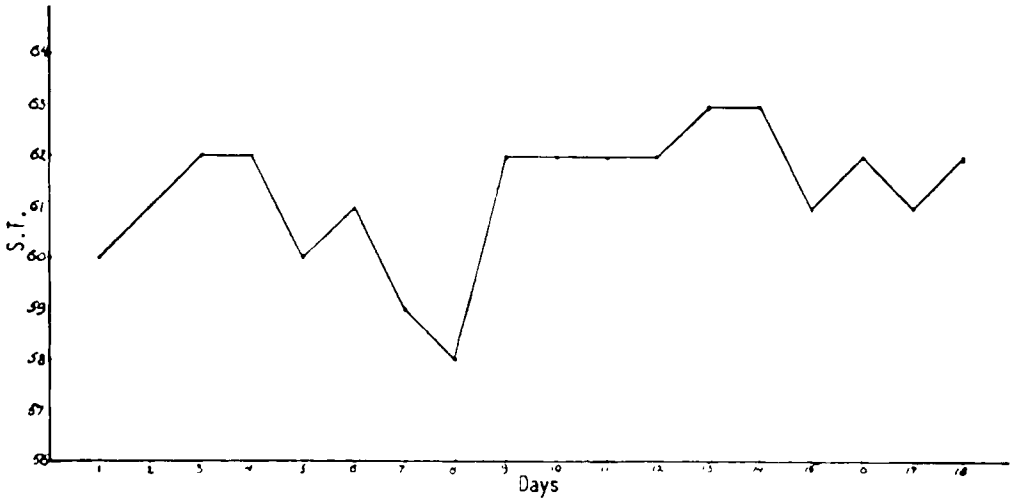

FIG. 224. Average value of surface tension of the urine in control mice over a period of 3 weeks.

The average surface tension readings in the control groups are shown in Figure 224. It can be seen that these values fluctuated in an irregular fashion between 58 and 63 dynes/cm. The surface tension values of the group maintained at 37°C show a steady sustained rise from 61 to 65 dynes/cm. *(Fig. 225)* The mice kept at 8°C showed an initial slight fall in surface tension, with a gradual return to the original levels. (225)

After several days in the incubator, the mice began to lose weight, their fur became sparse, and snout areas were constantly wet. The urinary output was scanty as compared with the two other groups. Death began to occur in the mice kept a high temperature on the 12th day. The animals in the refrigerator developed thick luxuriant coats and huddled closely together at most times. None died from exposure to this temperature.

The fact that the animals maintained at 37°C showed a steady rise in urinary surface tension was especially significant. As expected, the urine of these animals was scanty and more concentrated than for the other groups. With this diminution in volume, it would be expected that the concentration of surface-active substances would rise and the surface tension would be lowered. The fact that the exact opposite occurred indicates that the observed change has to be considered as an effect of the high temperature.

This appears especially interesting for the relationship between temperature and the two ST patterns. While higher temperature induces one

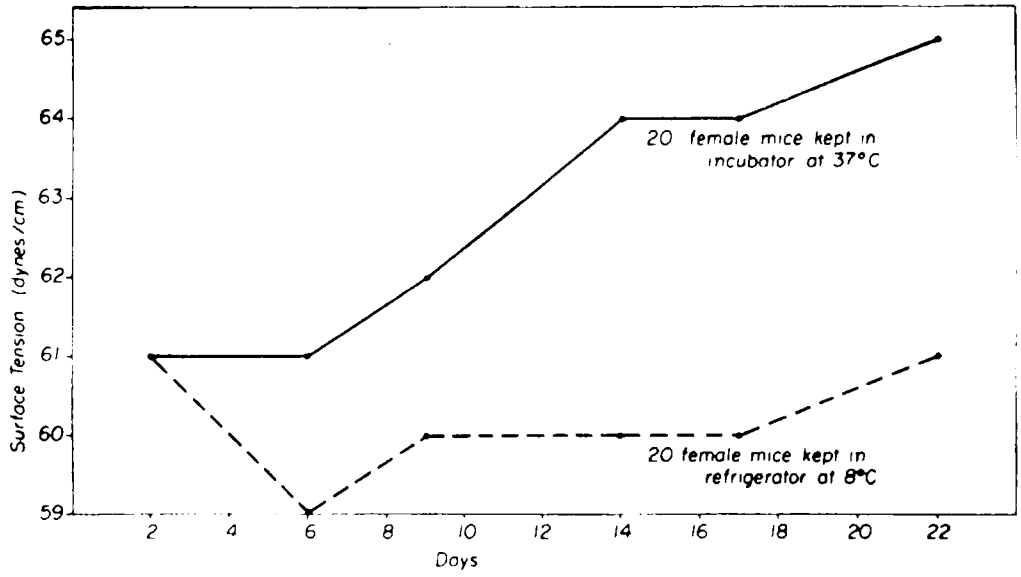

FIG. 225. The average values of the urinary surface tension of 20 female mice kept in the incubator at 37°C. and of 20 female mice kept in refrigerator at 8°C. The values are progressively increasing for the animals kept in the incubator until the animal died. For the animals kept in refrigerator, after an initial descent, the values ascend toward normal.

pattern, cold induces the other. It must be noted that the organism cannot defend itself against the pattern induced by higher temperature and the animal dies after a certain time, but for the pattern induced by cold, defense is possible. The body seems to be able to overcome the change. The surface tension returns to normal and the animal becomes adapted to the temperature. Not one of the animals kept in the refrigerator died, while all in the incubator were dead after a month. Adrenalectomy induces an immediate increase in the surface tension of the urine. *(Fig. 226)*

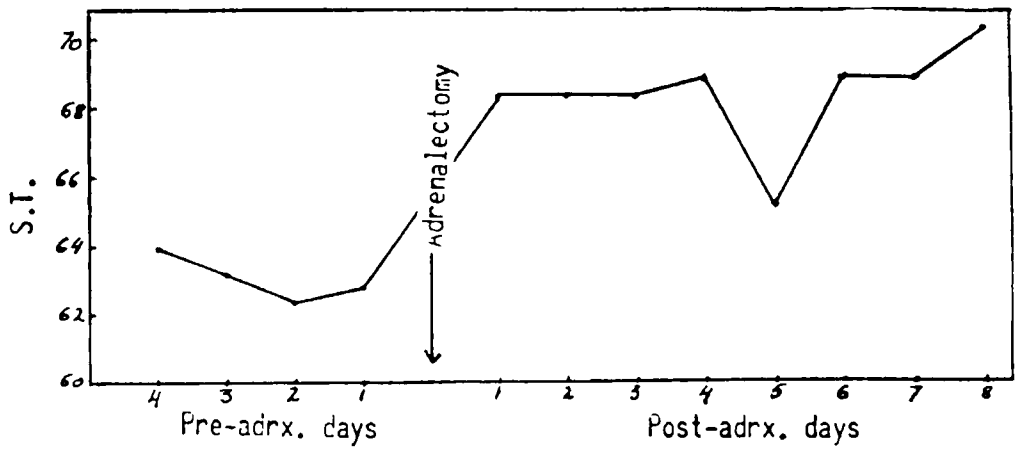

FIG. 226. The surface tension of the urine increases to high values after adrenalectomy.

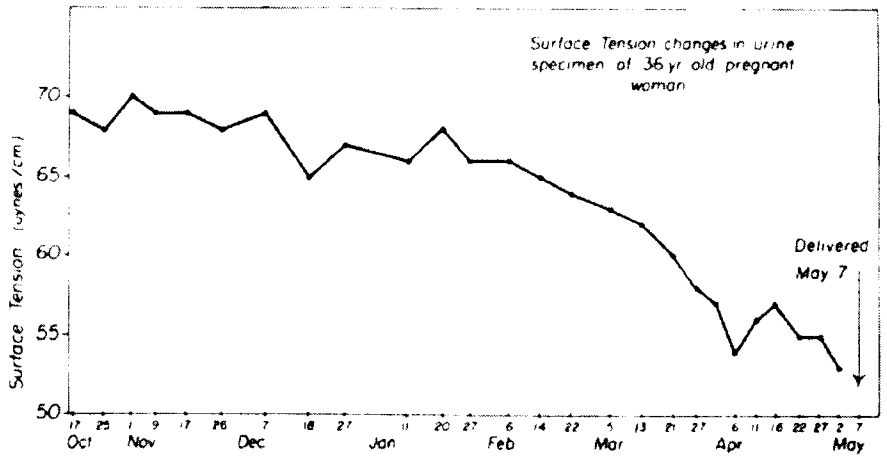

FIG. 227. Curve of the surface tension changes in urine specimens of a 36-yr. old pregnant woman shows a manifest change toward low values, starting with the 4th month, and becoming especially low in the last three months.

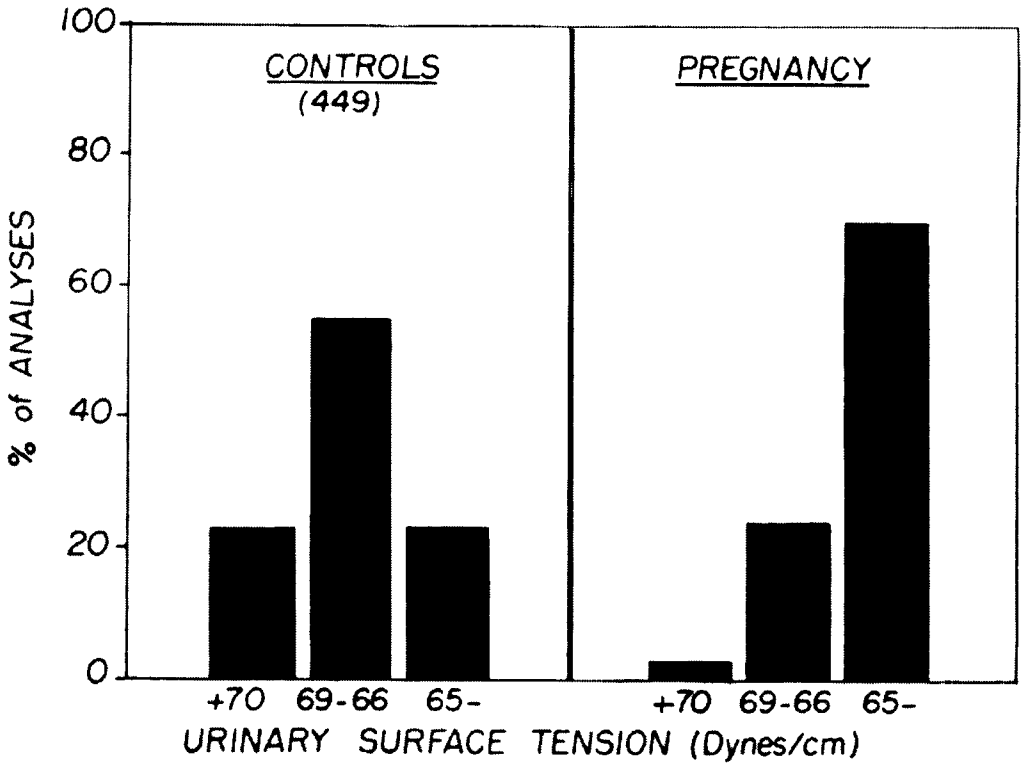

FIG. 228. The average value of the surface tension in pregnant women shows a manifest change toward low values.

Colloids in Urine and Surface Tension

An interesting relationship between urinary surface tension and the presence of "colloids" in the urine was noted by Butt and his associates. (240) Using the pendant drop method for the ST, direct examination in dark field for the presence of colloids in the urine, electrophoresis for determination of electrical charges, and the study of evaporated urine smears, they showed that urines with a high content of colloids, have a low ST; those with a low colloid content, a high ST. They have further correlated a low amount of colloids with a tendency of urine to precipitate and form stones. (242) They examined the colloid particles in the urine of different groups of individuals and found them high in Negroes, and especially high in pregnant women, which is in accord with the low surface tension of the urine which we found in these cases *(Figs. 227 and 228)* and the low tendency of both groups to form urinary stones.

We were interested in the relationship between variations in the urinary content of colloids and systemic patterns corresponding to high and low ST. R. Ravich in our laboratory has confirmed the correlation between presence of colloids and ST by using our urotensiometer. (219)

Chapter 4, Note 12. **Urinary Oxidoreduction Potential**

For the study of the *oxidoreduction potential* of the urine, we used a Beckman pH meter with platinum electrodes. We measured the potential at the pH of the sample and also at pH 7. For this purpose, the platinum and the respective calomel electrodes used for these measurements were introduced into the beaker of the Fisher titrimeter together with the electrodes of the potentiometer. After stirring, the pH of the sample and its oxidoreduction values were measured. The pH then was brought to 7 with HCl or NaOH solution, and the value of the oxidoreduction potential was again measured. Four values were thus obtained: the original pH, the titrimetric acidity or alkalinity, and the oxidoreduction values at the original pH and at pH 7. Figures 229 and 230 show a sample of such curves.

Chapter 4, Note 13. **Oxidoreduction Potential of the Urine**

We have tried to determine the oxidoreduction potential of urine samples by using the change of a color indicator in its leuco base. We chose toluidine blue which, with a rH_2 of 14, is at the middle of the scale of the rH_2 values. In order to eliminate two of the important factors which intervene in the oxidoreduction potential—differences in pH and temperature —we used a fixed temperature and very low pH. The degree of oxidoreduction potential was determined by the time necessary to obtain the discoloration for a standard amount of the color indicator. The reactive used was a solution of toluidine blue in a normal solution of hydrochloric acid. The amount was chosen so as to give a discoloration at 100 seconds for the normal individual. 1.5 cc. of a saturated solution of toluidine blue in al-

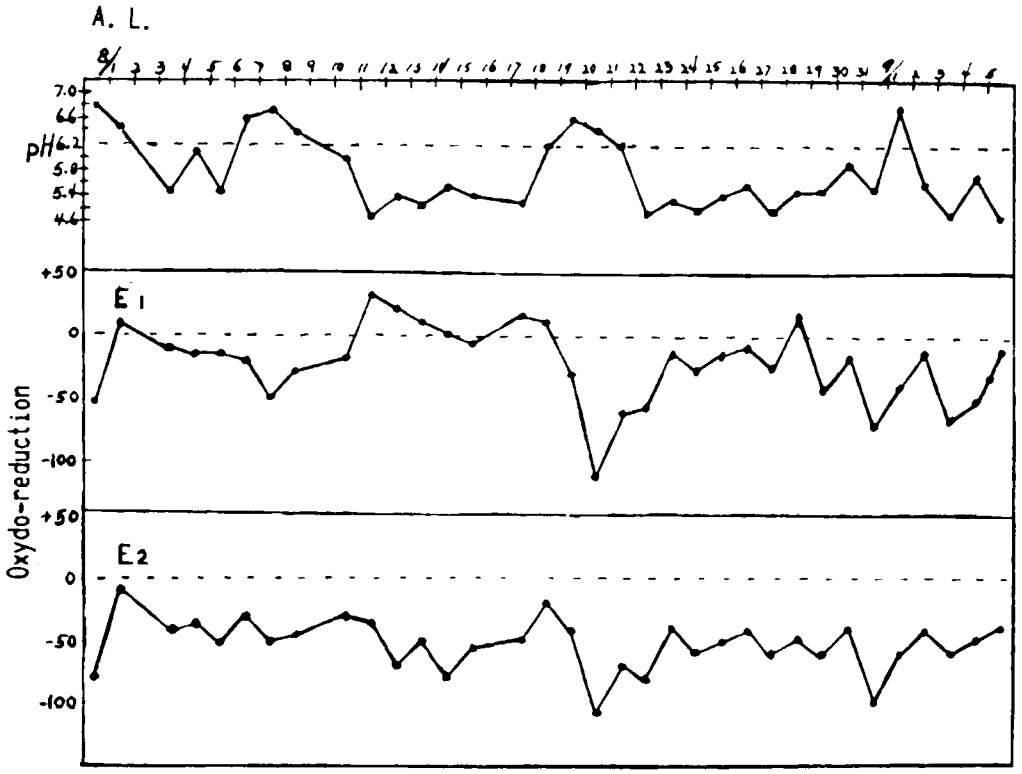

FIG. 229. The curve of the oxidoreduction potential of the urine measured electrically. The curve of the measurements made directly on the urine (El) show big variations which are smaller if the pH of the sample is brought to 7 (E2). In a case of cancer of the breast, the curve remains constantly below the 0 value.

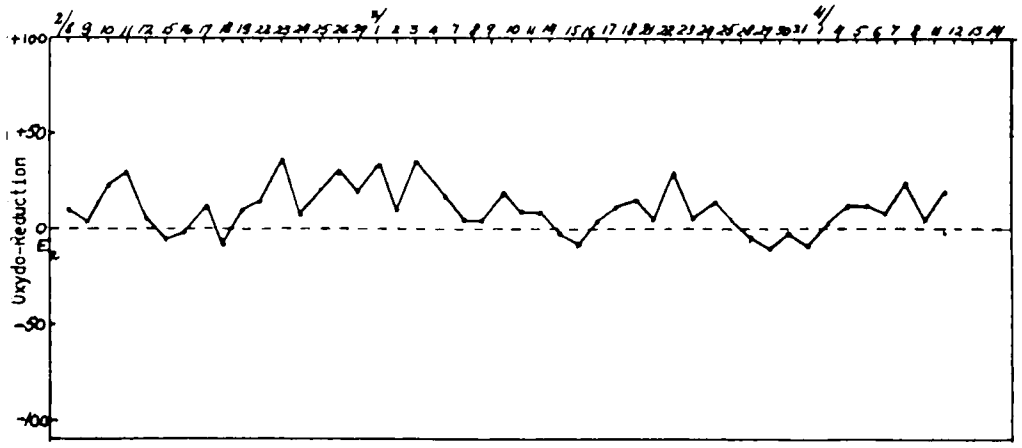

FIG. 230. Curve of the oxidoreduction potential values of the curve brought to pH 7 of a case of cancer of the breast, shows values around or above the 0 value.

cohol was added to 100 cc. of n/10 hydrochloric acid. 1 cc. of this reagent was added to 4 cc. of urine in a test tube kept for a while in boiling water. The time necessary for the discoloration was marked. Values as low as 3-4 seconds or as high as above 420 seconds were seen. A high oxidoreduction potential inducing a rapid discoloration was found to correspond to a pattern of the offbalance type A while a low discoloration was seen to corre-

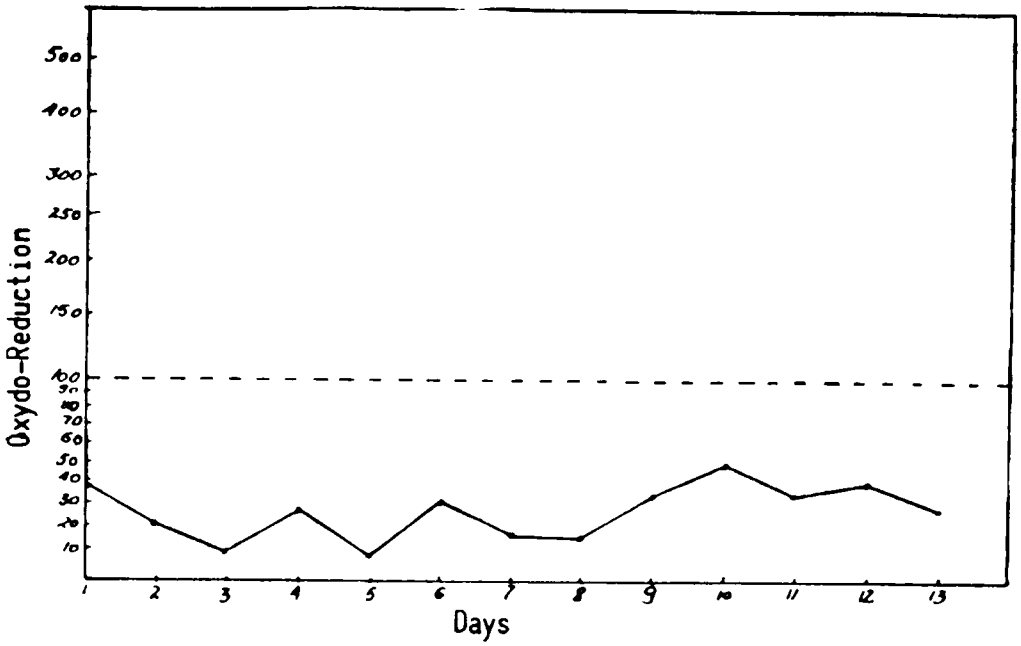

FIG. 231. Curve of urinary oxidoreduction values in a case of carcinoma of the breast with multiple bone metastases. The values are established as the time necessary to obtain the reduction at 100°C and with a pH around 2, of a solution of toluidine blue so chosen as to have 100 seconds as the average value for groups of normal individuals. In this case the values remain fixed low below 100 seconds, corresponding to a pattern, of the offbalance type A.

spond to the pattern of type D. Figures 231 and 232 show two such curves. We used this test for many years as main analyses to determine the existing offbalances. (220)

Chapter 4, Note 14. **Peroxides in the Urine**

The hypothesis of the existence of a phase "oxygen" of offbalance D led us to study the appearance in urine of products resulting from abnormal oxidation. We were especially interested in the existence of substances having peroxide properties. We found that addition of sulfuric acid to urine of certain subjects induced appearance of indigotin and indigo-rubin. In order to investigate the reaction, we have utilized the solubility of indigotin and indigo-rubin in neutral solvents. Through their extraction it appears possible first to prevent their transformation in colorless isatin and, second,

to evaluate the relative amounts when they appear during the reaction. To 4 cc. of urine, one centimeter of toluene was added. After shaking the mixture, 1 cc. of pure sulfuric acid was added and the mixture was immediately shaken again. When the mixture was allowed to stand, the toluene separated and its color, blue or violet, indicated the presence and also the relative amounts of indigotin and indigo-rubin.

Another method used to detect peroxides was the acidification of urine followed by addition of potassium iodide. For iodometric evalution, starch

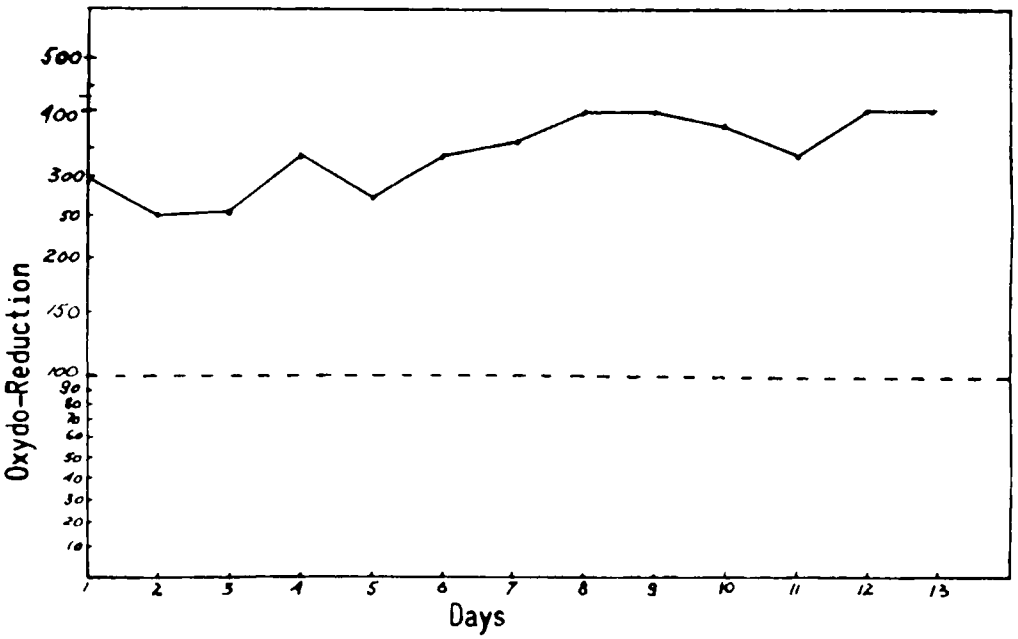

FIG. 232. The urinary oxidoreduction values in a case of cancer of the colon with abdominal metastases. The values remain the whole time above 100 seconds, corresponding to the pattern present in the offbalance type D.

solution was added. The amount of iodine liberated could be determined titrimetrically.

The form in which peroxides are present in the urine is not clearly established. Although the distillation of the urine gives peroxides in the first distillate, these are not in the form of hydrogen peroxide, since catalase does not induce their disappearance. The values obtained with both methods, sulfuric acid and iodometric, are relatively parallel. The sulfuric acid method, however, produced a higher percentage of positive results.

The presence of slight amounts of peroxide in the urine has been found in about 3% of normal subjects. In contrast, we found peroxide in the urine of 87% of a group of 27 schizophrenics studied through daily analyses over a period of three years. In some of the subjects, throughout the entire three-year period with more than 1,000 analyses, not a single negative reaction was seen. *(Fig. 233)* (221, 222)

We have also found positive reactions during streptococcic infection,

erysipelas or tonsillitis. In radiation sickness the reaction is positive especially when tissue lesions are manifest such as mucositis or epidermitis. In general, treatment with selenium has given a relatively high proportion of positive results, especially at the beginning of treatment. While positive reaction appeared to be consistent with a favorable evolution of tumors, an extremely intensive reaction appeared related to a bad prognosis. The following observation is characteristic.

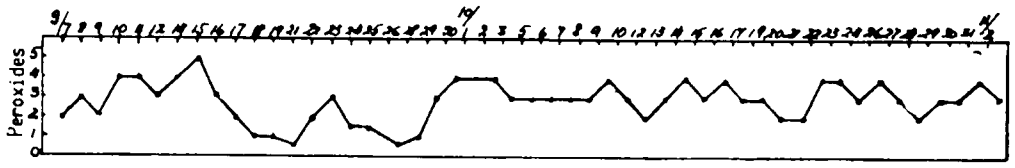

FIG. 233. The reaction for peroxides remains consistently positive in the urine of a schizophrenic in daily analyses during a period of 3 years. (Part of the curve.)

Mrs. N. C., 28 years old, with Hodgkin's disease, had received three treatments of teleradiotherapy, with the general condition completely unchanged. The patient presented an extremely intensive urine peroxide reaction. Dilution of the urine to 1/50 still showed a marked blue color after treatment with sulfuric acid and toluene. We informed the attending physician about the finding and advised the discontinuance, at least for the moment, of the treatment. The perfect general condition of the patient induced the radiologist to disregard our advice. A new treatment of teleradiotherapy was administered. Three hours later the patient, who only that morning had been shopping, went into a shock and died during the night.

We want to emphasize, however, the correlation between the lack of peroxides in the urine and a poor prognosis during radiotherapy. The cases in which a positive reaction disappeared, were always followed by a change for the worse in the general condition. The persistence of this lack of peroxides was seen in the cases with a rapid lethal termination.

Chapter 4, Note 15. **Index of Excretion and of Retention**

One of the most important aspects of the relationship of an entity to its environment is given by its intake and output. The concept of hierarchic organization with emphasis on the individuality of the entities, has given a special meaning to the study of these processes. For each entity its proper environment is represented by the secondary part of the entity immediately superior to it. The nuclear sap represents thus the environment from which the chromosomes take the material necessary for their metabolism and where they reject these substances which are no longer needed. Similarly, the cytoplasm represents the environment for the nucleus, the interstitial fluid for cells, the lymph for tissues, the blood for organs and the actual environment for the organism. This systematization, based on the organizational individuality of the entities, has guided the study of the relationship

between entities and their environments under normal and abnormal conditions. It is under this aspect that we have investigated the renal excretion, which according to the hierarchic organization, corresponds to the relationship between the organism as an entity and its environment.

Some of the substances excreted come from the metabolism of lower entities. Related to blood, they would represent often noxious undesirable substances, if the higher mechanism of the blood would not intervene. Although that which we see as urine is the result of the relationship between the organism as an entity and its environment, the origin of the different substances forming it, as related to the different other levels, has to be considered. While for certain elements this origin is evident, for many substances only suppositions are available today.

When a systematic analysis of these constituents was attempted, other difficulties arose. The isolated urine samples easily available, have only a very relative value for many of these investigations. The titrimetric data expressed as concentration of various substances are all functions of the amount of water eliminated in the sample. As this often varies widely, the informations obtained are only relative. Balance analyses concerning entire intakes and outputs represent such technical difficulties as to make them unavailable for routine investigation, in which hundreds of subjects are daily studied. We tried to bypass this difficulty by eliminating the factor water excretion, from the considered data. The fact that the concentration of a substance and the specific gravity of the urine, are both direct function of the amount of water present, has permitted to eliminate this factor. The ratio between them appears thus independent of the amount of water present. It relates the amount of a substance to that of the bulk of the substances eliminated through the kidney. An index of excretion was thus obtained by dividing the concentration of the substance by the specific gravity. The opposite ratio would correspond to an index of retention.

From the physiological point of view, these ratios are not affected by the factors which govern the glomerular filtration, which are acting similarly for all the substances present. They are little or not affected also by the back resorption, where the differences between the various substances are reduced. They will show consequently, big variations as resulting from the active reabsorption, which takes place in the distal portions of the convoluted tubes. It is this character which gives the indexes of excretion or retention, as we calculate them, their value. We have utilized for years these indexes for chlorides, sodium, potassium, phosphoric ion, sulfhydryl, calcium, to obtain valuable information which otherwise could not be furnished by the simple analysis of the isolated urine samples. We will come back to these indices during further analyses.

Chapter 4, Note 16. **Water and Nitrogen Metabolism**

The analyses of different urine samples have shown that the amount of water present in urine seems to influence indirectly its constitution. It could thus be seen that while very diluted urine, corresponding to a large

amount of water excreted, is usually alkaline, concentrated urine, corresponding to small amounts of water excreted, is generally acid. Furthermore, it could be seen that these changes are related to more profound metabolic differences. When related to the nitrogen metabolism, it could be seen that diluted urines are rich in free ammonia while concentrated urines in uric acid. We have thus investigated the relationship between these two factors, the amount of water excreted and the form under which the nitrogen is eliminated.

The comparative physiology shows us that the manner under which nitrogen is excreted varies for different animals according to the amount of water available in the surrounding environment. In fish, with water almost unlimited, nitrogen is excreted in the form of ammonia. The high toxicity in this form of nitrogen excretion is counter-balanced by the amount of water in which the excreta are diluted. Fish are ammonioselic. In terrestrial mammals, where the amount of water available is more limited, the excretion of nitrogen is made in the form of urea which is much less toxic than ammonia. The danger of poisoning the drinking water through excreta is thus reduced. Mammals are ureoselic animals. For birds, for whom water is scarce, the form of nitrogen excretion is of uric acid, which through its low solubility in water, has little chance to contaminate the drinking water. Birds are uricoselic. Based on this relationship between water availability and the type of nitrogen excretion, we looked for a similar relationship in humans between the excretable amount of water and the type of nitrogen metabolized under normal and abnormal conditions. An immediate confirmation was obtained in those abnormal conditions where the amount of water excreted is abnormal. As already seen above, in subjects having a high diuresis, which in general would correspond to a high amount of water available to be excreted from the body, the urine is usually alkaline, the alkalinity due to ammonia. On the opposite side of the normal, there are subjects with a very reduced urinary excretion. In patients eliminating only two to three hundred cc. in 24 hours, the amount of uric acid in the urine is manifestly increased. Upon standing, these urines always show a reddish deposit formed mostly by uric acid. Relating this to comparative physiology, while the normal subjects would appear ureoselic, those with polyuria can be considered as ammonioselic and those with oliguria, uricoselic.

We tried to see if a change in the form under which the nitrogen is eliminated can be induced by changing the amount of water available to be excreted. Normal subjects whose urines were tested for certain periods of time for their content of ammonia, urea and uric acid, were given 1 to 2 liters of water to drink. The highly diluted urine which was subsequently excreted, became alkaline. The total amount of ammonia increased while urea and uric acid were slightly reduced. The same subjects were later given a dry diet for 12 hours or more. The specific gravity of the urine in most of these cases was above 1.026, indicating a kidney with normal concentration capacity. Although the content in ammonia decreased manifestly in these urines, the increase in uric acid excreted was minimal. We sub-

mitted the same subjects to a diet with fluids reduced to a minimum, for 3 to 4 days. Under these conditions, the excretion of uric acid started to increase. Great intake of water would thus transform a normal ureoselic individual into an ammonioselic in only a few minutes. A similar passage into uricoselic was seen to need days, and even then it showed only minimal changes. This can be explained by the fact that while ammonia in urine appears largely through changes taking place in the kidney cells themselves, uric acid results from more profound metabolic changes, which concern especially the nitrogenous bases, particularly the purines.

Chapter 5, Note 1. **Second Day Wound Crust pH**

The metabolic processes characterizing abnormal foci have been investigated in various ways. One method was devised in order to study the acid-base changes which take place within abnormal foci created by surgically produced wounds under the influence of various chemical, physical and biological factors. This research was made in collaboration with C. Huesca-Mejia. (212)

Adult Carworth Farm female and male albino rats weighing 150 to 200 grams were employed. The animals were separated according to sex and all received a standard diet of Purina Chow and water ad lib. Tests were run on groups of twenty animals, with from two to four control animals in each group.

The animals were divided into groups that were subjected to various experimental conditions for three days, after which time, under ether anesthesia, a wide area of the back of each animal was carefully depilated by hand. A 1 square cm. wound was then produced in the depilated area down to the dorsal aponeurosis. The wound was kept free of blood with dry gauze until bleeding had entirely ceased, and was then left uncovered. All wounds were made between 8:00 a.m. and 10:00 a.m.

A glass electrode made according to the specifications of McInnes and Dole (223) was used. As reference, an electrode containing normal saline was employed. (224) The electrodes were mounted on a stand in a fixed position. A model H Beckman pH meter, whose signal was amplified by a 6H6 and 6SN7 push-pull, was used as the first amplifier. The readings were made on a 200 micro-ampermeter adjusted so as to have the full scale represent one pH unit. The experimental error using this apparatus was found to be less than $\pm$ .01.

pH determinations were carried out by bringing the wound area into contact with the tips of the electrodes. The animals were held gently but firmly in one hand until they were completely immobile, at which time a firm contact was established between the center of the wound area and the tip of the glass electrode, and between the reference electrode and the wound periphery. Readings were carried out on the surface of the freshly exposed aponeurosis within ten to fifteen minutes after the surgical procedure. Subsequent readings were made every twenty-four hours upon the wound crust, which was washed and then moistened with a drop of an

0.1% sodium chloride solution freshly adjusted to pH 5 as suggested by Blank for use in skin pH determinations. (225) The electrodes were standardized with buffers of different pH before and during each period of testing.

The effect of various chemical agents upon the wound pH was studied by daily oral administration for three days preceding the operation and thereafter until the conclusion of the series of pH determinations. In general, the substances were administered according to weight in quantities corresponding to the therapeutic doses accepted for humans or in amounts equal to 10% of the daily toxic dose. Water soluble substances were administered in drinking water. Oil soluble substances were administered on pieces of bread. In some cases, substances were introduced directly into the stomach through a catheter. In a few cases, substances were injected subcutaneously.

In the 164 control animals, the pH of the wound surface varied between 7.30 and 7.33 when measured ten to fifteen minutes after the completion of the surgical procedure. The pH remained at this level for at least three hours.

Twenty-four hours after the wounds were produced, the pH of the moist crust covering the area was found to be between 7.72 and 7.76 in all untreated animals. *(Fig. 234)*

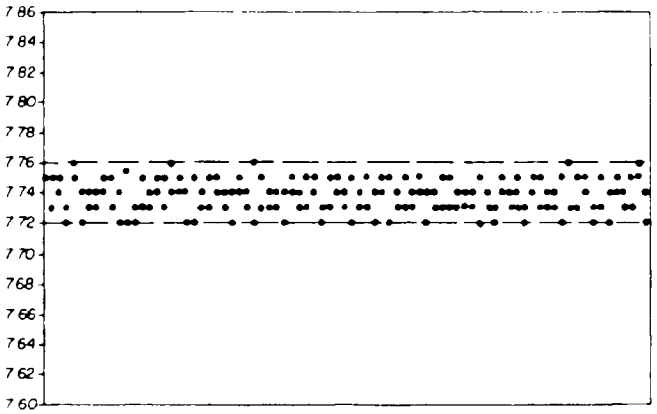

FIG. 234. Second day wound crust pH values remain in a range from 7.72 and 7.76 in normal rats as seen in the animals which served as controls for the multiple experiments.

At forty-eight hours, the pH of the crust was found to be between 7.42 and 7.60, this being the period of greatest variability. At seventy-two and ninety-six hours, all the readings were between 7.28 and 7.32. After the fifth day, similar values were found and further readings were not taken.

No consistent correlation was found between the values on different days for individual animals. For example, in some animals with a wound pH of 7.30—the lowest value—immediately after the operation, pH reached 7.76, the highest value for the normal range, in twenty-four hours. In males, values appeared to be slightly higher than in females.

In 410 of the 860 animals exposed to various chemicals pH value of the wounds was determined several minutes after the operation and found to be between 7.28 and 7.35. Only 22 showed a deviation from the control range of 7.30 to 7.33. No correlation was observed between the minimal changes in these animals and the type of treatment administered.

While the pH values found several minutes after wound induction showed very little or no variation from the control range and no correlation with the various experimental conditions employed, the findings at twenty-four hours showed considerable significance for the agents used. At forty-eight hours and thereafter, no important differences were observed between values for controls and those for groups treated in different ways. In continuing these studies therefore, determinations were carried out only several minutes after the production of the experimental wounds and then again 24 hours later. Actually, only values for the twenty-four hour reading appeared significant and will be discussed. We made 24 hour measurements for all of the 860 animals treated with different agents. For convenience, we refer to the pH value of the crust at twenty-four hours after creation of the experimental wound as the s.d.c. pH (second day crust pH).

From four to twelve animals were employed in the assays of the activity of each agent. By applying the same experimental conditions to animals in groups tested at different times, it was possible to determine whether the changes observed were due to some external factor such as temperature, humidity, etc., or were actually due to the imposed experimental conditions. The s.d.c. pH has proven to be of considerable interest because consistently similar changes have been found to be produced by the same agents when applied to animals in different groups tested weeks or months apart.

Considering all the animals treated with various agents, three possibilities have been found to exist: 1) There may be no effect upon the s.d.c. pH, in which case the values will all fall within the control range of 7.72 and 7.76 found in untreated animals; 2) s.d.c. pH may be elevated to values between 7.77 to 7.85; or 3) the s.d.c. pH may be reduced to values of 7.70 to 7.60.

We will present here only the conclusions of these studies as related to the various agents investigated.

CHEMICAL FACTORS

Cations and Anions

It was interesting to investigate the influence exerted by some cations and anions by first using the same anion with various cations and then using different anions with the same cation. It was apparent in all the experiments that the immediate pH of the wound does not differ from that of the untreated animals, and that the s.d.c. pH data obtained are concordant.

We studied the influence exerted by anions first by investigating the effects of administration of acids. With even strong inorganic acids, no