A CLINICAL METHOD OF HAEMALKALIMETRY, WITH APPLICATIONS TO DETERMINATION OF THE REACTIVITY OF THE INORGANIC SALTS OF THE SERUM IN MALIGNANT DISEASE AND OTHER CONDITIONS

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(Received May 16th, 1906)

The experiments recorded in this paper were commenced with the object of determining the alkalinity of the blood in malignant disease.

Our attention was attracted to this subject in consequence of the observation that secretion of hydrochloric acid in the gastric juice is diminished or suppressed whatever be the situation of the malignant growth, which points to the probability of an increased alkalinity of the plasma in malignancy.¹

The determination of the reaction of the blood and its degree of alkalinity or acidity, is a subject surrounded by great difficulties, both experimental and theoretical, as is shown by the great number of methods which have been introduced for the purpose, and the great divergencies in the results obtained.

The experimental difficulties are introduced by the colour and composite nature of the fluid, the presence of the proteins, which react as acids or bases, according to the indicator used to point out the neutral point, and the peculiarly balanced phosphates and carbonates of the alkali metals present which act in a similiar manner.

The older experimental results vary according to whether the reaction is taken in the whole blood or in the plasma or serum, and still more according to the indicator used by different observers.

1. This Journal, preceding paper, p. 274

The theoretical difficulties are caused by a general haziness, which surrounds the literature of the subject as to what constitutes a neutral reaction in a fluid. It is only recently that advances in physical chemistry and improvements in methods of determining ionic concentrations have begun to shed light on the results of determinations, by means of coloured indicators, of what was formerly supposed to be the alkalinity or acidity, and to show that the reaction lies not very far removed from the neutral point.

It by no means follows from this, however, that titrations with coloured indicators are valueless, and should be abandoned in favour of such measurements of ionic concentrations as can at present be carried out by physical methods.

In the first place, although the titrations do not give the true neutral point, and therefore the degree of alkalinity or acidity of the blood or serum, they do give something which is of high value in physiology and pathology, namely the amount of alkali on the one hand, and acid on the other, which can be added to these fluids without raising the hydroxyl ion concentration or hydrogen ion concentration above certain low limiting values.

This property which is related to the reaction, and yet, in value may be quite different from the reaction, requires a special term, and has been called, in the previous paper, the *reactivity*.

As has been pointed out in that paper, a long range of reactivity is essential to the normal physiological life of the cell, once its limits have been passed, striking pathological changes occur, and with a slight further increase in either hydroxyl or hydrogen ion concentration, life becomes impossible.

The peculiar content of the plasma, and of the cell protoplasm also, in proteins, and in carbonates and phosphates of the alkali metals, increases the range of reactivity, or in other words, the amount of alkali or acid which can be set free in the body without causing the hydroxyl or hydrogen ion concentration to rise so high as to disturb or prevent the metabolic processes in the protoplasm which are necessary for life.

Further, the limits of variation in the ratio of the concentrations of the hydrogen and hydroxyl ions which are compatible with life at all, and certainly with life under physiological conditions, are so narrow as to render the variations in these concentrations impossible of determination by any of the methods at present known in physical chemistry.

For example, the blood plasma or serum gives the acid indication to the indicator, phenol-phthalëin, and the alkaline indication to the indicator methyl-orange. Now if either sufficient alkali were added to give the alkaline indication to phenol-phthalëin or sufficient acid to give the acid indication to methyl-orange, then the evidence of experiment on the effect of such changes upon living cells leads us to conclude that life, as a result of such changes, would become quite pathological if not altogether impossible.

Thus, in the case of the developing fertilized egg of *Echinus esculentus*, it has been shown that a concentration of $\frac{1}{750}$ normal of either acid or alkali completely stops growth and cell division, and quickly kills the cells.

This illustrates the narrowness of the limits of change in hydrogen and hydroxyl ionic concentrations which are compatible with life, and the necessity of a fluid constituted like the plasma, for preserving the concentrations within physiological limits. Without such a conserving agency any small liberation of acid or alkali in the body would lead to profound disturbances of the metabolic processes.

It is just within these narrow limits of variation in the relative values of hydrogen and hydroxyl ionic concentrations lying around equality, that physical methods of determining ionic concentrations at present leave us without assistance, and where it is, therefore, of value to retain titration methods when read in the light of the knowledge given by the physical methods. For although the titration methods do not give the ionic concentrations, if it can be taken as approximate that the fluid contains carbonates, phosphates, etc., in about the same relative proportions in different cases, then the titrations will be roughly proportional amongst themselves to the ionic concentrations, because they indicate the total concentration in these salts, and upon this the hydrogen and hydroxyl ionic concentrations ultimately depend.

With such an imperfect method we are, at any rate, forced to be content until increased sharpness can be given to physical methods for determining hydrogen and hydroxyl concentrations in such a fluid as blood plasma or serum.

For example, it is quite impossible to determine the concentration of hydroxyl or hydrogen ions in the plasma by the hydrolysis method, and with hydrogen electrodes it is impossible to get any nearer than that the concentration of the hydrogen ions approximates to their concentration in distilled water. Now the results of different observers vary by 0.5×10^2 for the value of the hydrogen ion concentration. This means that the concentration of the hydrogen ions might be fifty times that of the hydroxyl ions or, conversely, the concentration of the hydroxyl ions fifty times that of the hydrogen ions without the possibility of detecting a difference by the method. The living cell is so extremely susceptible to variations that it is just in this immeasurable zone that all the physiological interest lies.

Although the recent observations show that the reaction of the serum lies, approximately, at the neutral point are of high interest, such knowledge alone is insufficient, and what is wanted is accurate experimental knowledge of the small amount by which the reaction normally differs from neutrality, and of the small variations which occur in diseased conditions.

The only course, at present available, is titration under uniform conditions, as free from experimental objections as possible.

For this reason we have carried out titrations of the serum to (a) phenol-phthalëin and (b) di-methyl-amido-azo-benzol (called afterwards 'di-methyl' for brevity); and also titrations of the inorganic salts of the serum after removal of the proteins by incineration. Such determinations have been made (a) in healthy individuals of both sexes, (b) in persons debilitated by various diseases or by accidents, and (c) in persons suffering from malignant disease.

Before passing, however, to a description of our methods and the results of our experiments, it will add to clearness to make some preliminary statements as to neutrality to different indicators, its meaning, and its relationship to neutrality as indicated by physical methods.

The statement is often made that the blood (or plasma or lymph) is a fluid which is at the same time acid and alkaline, or at one time the acidity of the blood is spoken of, and at another its alkalinity.

Such statements are, however, meaningless and absurd, the blood cannot at the same time be both alkaline and acid, for the two properties are diametrical opposites of each other. Between acidity and alkalinity there exists a neutral point, and any given solution *must* lie to one side or other of the neutral point, that is to say, it must be either acid or alkaline.

The statements arise from the fact that many fluids (including the blood and other body fluids) show the acid colour or indication to one coloured indicator and the alkaline indication to another indicator.

The statement would, therefore, be somewhat less objectionable that a given solution is acid or alkaline to a stated indicator; for example, the blood is acid to phenol-phthalëin, and alkaline to litmus, methyl-orange and 'di-methyl,' or the urine is acid to phenol-phthalëin and litmus, and alkaline to methyl-orange and 'di-methyl.'

Even this method has the objection, however, that it introduces the conception that one and the same solution has several different neutral points, and that, therefore, the neutral point is a variable thing dependent upon an artificial colouring agent which is merely introduced to discover or show a supposed neutral point existing in the solution before its addition.

The questions may be raised—what is the meaning of all these different neutral points, is there any single definite neutral point not dependant upon indicators, if so, has it any relationship to the various neutral points given by coloured indicators, and, finally, what physiological interest do the reactions to coloured indicators possess?

All acid solutions contain hydrogen ions in excess, and all alkaline solutions contain hydroxyl ions in excess, which at once suggests that the true neutral point between acid and alkali is that point at which the concentrations in the solution of hydrogen and hydroxyl ions are equal.

This condition is realized in pure distilled water, for here the only source of either hydrogen or hydroxyl ions is the dissociation or ionization of the water molecules themselves, and hence the number of the hydrogen and hydroxyl ions must be equal.

The condition of equilibrium in the water may be represented by the equation :—

The excessively low conductivity of pure distilled water shows that the number of conducting ions is very low compared to the non-ionized or water molecules.

If now to the water there be added a *trace* of acid, this is almost completely ionized, and one of the ions is always a hydrogen ion, as a result of this (remembering that the water is practically non-ionized and the added acid almost completely ionized) the hydrogen ion concentration is enormously increased. Also the hydroxyl ion concentration falls in the same ratio, so that the hydroxyl ion concentration is almost reduced to zero. This follows because the *product* of the hydrogen and hydroxyl ion concentrations remains practically constant, and, therefore, as hydrogen ion concentration goes up, hydroxyl ion concentration correspondingly falls, so as to keep the product constant.¹

As a result of these two factors acting in the same direction it follows that the ratio of hydrogen ion concentration to hydroxyl ion concentration, even in a very dilute solution of an acid, goes up enormously. Similarly in a very dilute solution of a caustic alkali the hydroxyl ion concentration rises and the hydrogen ion concentration falls so that the ratio becomes very high.

As a result even a very dilute solution of free acid or alkali shows most markedly the corresponding acid or alkaline properties. The position is quite different when to the distilled water are added such salts as the acid carbonates and phosphates. Here the ratio of hydrogen and hydroxyl ions varies incomparably more slowly, and,

1. If CH_2O , CH , and CHO represent concentrations, then from the equation of equilibrium of the water and its two ions given in the text, we have, from the mass action law, the equation $K_1 \text{CH}_2\text{O} = K_2 \text{CH} \times \text{CHO}$, where K_1 and K_2 are two constants. but CH_2O , the concentration of the water-molecules for dilute solutions is almost constant also, from which it follows that $\text{CH} \times \text{CHO}$ is approximately constant.

according to the relative proportion of acid and base, the hydrogen or hydroxyl ions respectively may be in excess.¹

The cause of this is that the added acid salts such say as NaHCO_3 , NaH_2PO_4 and Na_2HPO_4 yield on hydrolysis by the water and ionization both hydrogen and hydroxyl ions and not one of these only, so that there is no union of two factors running one concentration up and the other down as in the previous case, but two opposing tendencies which partially balance each other and come into equilibrium.²

It is for this reason that the dual reaction to different indicators shown so markedly by blood serum (and many others of the body fluids) arises, and this is also why such a dual reaction is practically never seen with solutions of free acid or alkali even at extreme dilutions.

To understand how this dual reaction arises, and why as a result the same fluid appears to show just as many different apparent neutral points as indicators are used for its titration we must consider briefly what coloured indicators are and how they act. We shall see that they really do not indicate the neutral point but rather a point at which there is a certain definite degree of dissociation or ionization of the indicator itself that this point corresponds to a *comparatively* low ratio of hydrogen and hydroxyl ions, but a ratio which is different for each particular indicator. Also, because the ratio of the hydrogen and hydroxyl ions alters very slowly in a solution of bi-carbonates or phosphates the neutral points to the several indicators are drawn widely apart; while because the ratio varies enormously rapidly when free acid or free alkali is being titrated the neutral points to the different indicators are crowded together so that practically they come to the same point as far as can be experimentally observed, and thus the solution does not indicate acidity to one indicator and alkalinity to another.

The coloured indicators used for titration in alkalimetry consist of organic substances which possess the property of acting as very weak acids or very weak bases, or of salts of such feeble organic acids or bases, further, in solution these become partially ionized and the organic ion resulting from their ionization is coloured and has a different

1. It is not meant by this that the two concentrations are nearly equal; the ratio may be 100 to 1, or 1000 to 1, while with an equal amount of added free acid or alkali it might be 100,000 to 1 or 1,000,000 to 1.

2. See previous paper.

colour from the non-ionized molecule of the salt or organic base or acid which is usually also coloured.

The so-called neutral point is that point at which the concentrations of organic ion and of organic non-ionized substance, in the form of salt, acid or base, as the case may be, are so balanced that the colour is the colour of passage between the colour of the ion and the colour of the non-ionized molecules of the indicator.

The neutral point is hence given by an equality in physiological intensity of two different colour tones blending into the intermediate colour, and it is important that the two colours shall be as distinct as possible so that the physiological effect of the change from the one to the other shall be striking and easily visible. One interesting result of this is that the neutral point varies for different individuals somewhat, so that a series of titrations to be comparable should be made by the same observer, in the same kind of light, and the observer's colour sensations should be sharp and as normal as possible.

It is clear from the above alone that the so-called neutrality of a solution need not coincide with the point at which the concentrations of hydrogen and hydroxyl ions are equal.

As a matter of fact the point of neutral colour *never* lies at the point of such ionic equality, the ratio of concentration of hydrogen and hydroxyl ion concentrations varies for each indicator, and in the case of any indicator varies also within narrower limits with the concentration of the indicator in the solution, so that it becomes important in any series of titrations to keep the concentration of the indicator constant (compare series below), and also to use the smallest possible concentration of indicator compatible with a clearly observable change in colour.

The ratio of concentrations of hydrogen and hydroxyl ions at the neutral point of colour is always however, a relatively low one compared to that obtaining in even a very dilute solution of any strongly ionized free acid or alkali, and it is upon this fact that the use of indicators for titrating acids or alkalies depends.

The changes in colour in a solution containing an indicator caused by addition of alkali or acid are due to the fact that the

ionization of the salts of these weak organic acids or bases, are different from the ionization of the free organic acid or free organic base. Suppose the indicator is a weak organic acid (as is the case, for example, with phenol-phthalëin or litmus), then addition of excess of alkali will cause the formation of the salt of this acid which is largely dissociated in solution into its ions hence the solution will have the colour of the organic ion (blue in case of litmus, pink in case of phenol-phthalëin). If, on the other hand, acid be added in excess to the solution containing the indicator then the organic acid is set free in the solution instead of the salt, now this organic acid is scarcely dissociated, hence the concentration of the organic ion falls almost to zero and the concentration in molecules of non-ionized indicator rises. As a result the colour of the solution now becomes that of the non-ionized indicator molecules (red in the case of litmus, colourless in the case of phenol-phthalëin). Intermediate between these two points of acidity and alkalinity, there is a point where the two colours of ionized and non-ionized indicator molecules are equal in intensity (or in cases of one colour only where the colour just appears or just disappears) this is the point of neutral colour to the indicator—the so-called neutral point—but it is obvious such a point need not lie at equality in concentrations of hydrogen and hydroxyl ions. Its exact point depends upon those ratios in hydrogen and hydroxyl ions, which, at the given concentration of indicator in the solution, keep it dissociated to that point at which coloured ion and coloured non-ionized molecule of the indicator balance in physiological intensity of colour.

The above reasoning may be made clear by reference to a weak organic acid and one of its salts which are not coloured, but in which the change in ionization can be shown by other physical methods. As such an example we may take a dilute solution of acetic acid and compare it with a solution of sodium acetate of equal molecular strength. In such solutions there is an equilibrium between the ions and the non-ionized molecules which may be expressed by the equations :—

As shown by the electrical conductivity and other physical methods, there is a great quantitative difference in the ionization in the two solutions, for the acetic acid is almost all non-ionized, that is in the condition shown on the left hand side of the equation, while the sodium acetate is strongly ionized and is hence chiefly in the condition shown by the right hand side of the second equation. Suppose now the non-ionized acetic acid (CH_3COOH) or its sodium salt (CH_3COONa) had, say a blue colour, and the acetyl ion (CH_3COO^-) had a red colour, then when in the condition of acetic acid with low ionization the colour would be blue, with scarcely any red which would be concealed by the excess of blue, and if alkali were added it would pass finally into red as the sodium acetate became more and more ionized giving more and more of the red coloured acetyl ion. Conversely, if the solution at first contained highly ionized sodium acetate it would be red in colour, the relatively highly concentrated acetyl red coloured ion out-balancing the small amount of non-ionized blue coloured sodium acetate, and if any acid were added, acetic acid would be formed, which, being but feebly ionized, would give the blue colour of the non-ionized molecules present in excess, and intermediately the neutral tint of purple would be passed through.

It is clear that the neutral point with indicators corresponds to equal physiological intensity in the two colours which are blending, this in turn depends on a certain definite ratio in the concentrations of coloured ion and coloured non-ionized molecule. There need not be equality, in the physical sense, of these two concentrations, and still less need there be equality in the concentrations of hydrogen and hydroxyl ions producing that given ratio of dissociation in the indicator which is responsible for the colour balance.

The ratio in the hydrogen and hydroxyl ions at the point of balance in colour is dependent upon the ease of ionization of the indicator, and this in turn upon the chemical constitution of the indicator and its concentration in the solution.

The same reasoning applies to a coloured base or its salt used as an indicator, except that here the feebly ionized free base is formed by addition of an alkali, while the strongly ionized salt is formed on

addition of an acid. But as before the non-ionized molecule of the indicator must be different in colour from the organic ion of the indicator.

In order that a colour base or colour acid may make a good indicator, it is necessary that the neutral colour points shall correspond to a low ratio in hydrogen and hydroxyl ions, or otherwise it will only react at all with strongly ionized acids or alkalies, and also that the degree of ionization of the indicator shall change rapidly with the change in ratio of hydrogen and hydroxyl ions at the neutral colour point otherwise the colour change will not be sharp.

The sharpness of colour change also depends upon the ionization of the alkali or acid being added, for the more completely ionized this is the less of it will have to be added to cause sufficient change in hydrogen and hydroxyl ion concentrations to induce perceptible changes in ionization of the indicator and so change the colour.

It is clear that an indicator which has its neutral colour point at a low concentration in hydrogen ions is most suitable for titrating weak acids, such as carbonic acid and the higher fatty acids (oleic, palmitic, etc.), for such an indicator will change at an exceedingly low concentration in hydrogen ions. Such an indicator is phenol-phthalëin, and it is for this reason that serum gives an acid reaction to it on account of the excess of carbonic acid which the serum contains. On the other hand, indicators which have their neutral colour point at a comparatively high concentration in hydrogen ions, such as methyl-orange and di-methyl-amido-azo-benzol, do not react at all to carbonic acid and other acids of low ionization, because the concentration in hydrogen ion produced by such acids is never high enough to produce the neutral colour point. It is just such indicators which react best with weak bases, because their neutral colour point corresponds to comparatively high hydrogen ion concentration and, therefore, low hydroxyl ion concentration, and hence in presence of weak bases the colour reaction remains on the alkaline side until a sufficient amount of strongly ionized acid, such as a mineral acid, is added to first combine with the weak base and then rapidly raise the hydrogen ion concentration until the neutral colour point is reached. It is for this reason that

serum is strongly alkaline to methyl orange or 'di-methyl,' and require enough acid to be added to satisfy the combining power of the proteins of the plasma, which act as feeble bases, before the colour changes to show an acid reaction.

Before passing from this brief outline of the modern theory of indicators, in so far as it concerns the interpretation of our results, it may be of interest to point out, since it has also a bearing upon our results, that the proteins of the cell protoplasm, behave to changes in hydrogen and hydroxyl ions in a manner exactly similar to indicators. They are, indeed, substances with feeble alkaline or acid properties which are much more sensitive to changes in hydrogen and hydroxyl ions than are most of the coloured indicators, and all that has been said above about indicators applies equally to them, and is on this account of physiological interest.

The changes produced by alterations in concentration in hydrogen and hydroxyl ions in the living cell are not shown as in the case of indicators by obvious colour changes, but they are none the less obvious, being shown by marked alterations in metabolism, growth, and nuclear division.

The cell is constituted so that it can only carry out its functions, normally, in presence of certain concentrations in hydrogen and hydroxyl ions lying not far removed from equality. It is provided against any marked alteration by a nutrient fluid which prevents sudden changes in the concentrations of these ions, and the composition of the nutrient fluid in this respect is kept delicately regulated by the excretory mechanisms and compensatory metabolic processes, so that it is exceedingly difficult, in the more highly organized animal, to cause, by artificial means, changes in the hydrogen and hydroxyl concentrations to which the cells are subjected.

To summarize, determinations by physical methods of the hydrogen ion concentration in blood or blood serum show that the hydrogen and hydroxyl ion concentrations lie comparatively near together, but such physical methods are not sufficiently delicate to give the small variations of the ratio in hydrogen and hydroxyl ion concentrations in this region, or to demonstrate such alterations in the

ratio as may occur in diseased conditions. Treatment of cells growing in an isolated condition, where there is no compensatory mechanism, to small variations in hydrogen and hydroxyl ion concentrations shows that these are most susceptible to such variations. Although titrations of the serum do not directly give variations in the ratio of the ionic concentrations, they do show the range of acid or alkali which can be added without the respective concentrations passing certain limits; they also show, at least qualitatively, by increased or diminished titration values that the ratio has changed, and give a rough quantitative index to the amount of change.

For these reasons we have carried out the series of experiments given below, and since other work, already mentioned, had led to the view that alterations might be expected in malignant disease, we have given special attention to determinations of the alkalinity of the serum in a series of malignant cases.

The serum has been used in preference to the whole blood, because, as pointed out by Wright,¹ it possesses the following advantages:—(1) The red blood cells do not interfere, and (2) from a clinical point of view the alkalinity of the serum is more important, because it comes into such close contact with the tissues, and may be taken as an index to the changes taking place in the circulating blood.

It may be pointed out, however, that in the second of the two methods, described below, in which the alkalinity is determined after removal of organic matter by incineration, the whole blood can be used, and we have found by experiment that accurate results can be obtained by the method when the whole blood is taken.

At the outset of the experiments the entire method described by Wright was employed, using litmus paper as an indicator, but it was soon found that reliable results could not be obtained with litmus, because four or five consecutive dilutions of normal acid gave the same colour reaction before a definite acid reaction was reached. This colour varied from pale blue on the outer edge of the moist circle of

1. *Lancet*, 1897, Vol. II, p. 719.

paper made by the drop to pink in the centre, so that it was difficult to decide which dilution corresponded to the neutral point.

Moreover, in addition to these experimental disadvantages, neutrality to litmus does not correspond to any definite point in the titration of carbonates and phosphates, giving neutrality with carbonates when there is a certain excess of carbonic acid above that necessary to form bi-carbonate, and neutrality with phosphates, at an intermediate point between the primary phosphate (MH_2PO_4) and the secondary phosphate (M_2HPO_4).

The indicators phenol-phthalëin and di-methyl-amido-azo-benzol were, therefore, substituted for litmus. The former of these gives neutrality with carbonates almost exactly at the point where bi-carbonate alone is present, and with phosphates at the point where secondary phosphate (M_2HPO_4) alone is present; while 'di-methyl' changes colour at the point where all carbonate is neutralized, and where phosphate is present as primary phosphate (MH_2PO_4). The use of these two indicators, therefore, correspond to fairly well-marked points. The colour change to both these indicators is much better marked than to litmus, so that sharper readings can be obtained.

For obtaining the serum, measuring its quantity, and for the process of neutralizing it, the method of Wright has been closely followed.

In the case of the 'di-methyl' titrations a very interesting and high value was obtained for the 'reactivity,' which showed that the proteins of the serum acted as bases, and were completely combined with acid before this indicator reacted. The figure obtained for the reactivity of normal serum was 0.170 to 0.180 normal, this indicates in addition to the reactivity of the inorganic salts, the reactivity of the proteins or in other words their power of combining with acids. The reactivity of the inorganic salts alone after the removal of the proteids was found to be 0.030 to 0.038. Subtracting these figures from the above values for reactivity of proteins, plus inorganic salt, the value of the reactivity for proteins alone is obtained as 0.140 to 0.142 normal.

It may be pointed out how close this value lies to the concentration in entire inorganic salts of the serum as shown by ash analyses or depression of freezing point. The value of the concentration of the total inorganic salts of mammalian-serum is equivalent to that of a 0.9 per cent. solution of sodium chloride, this expressed as a fraction of a normal solution amounts to 0.15 normal. It looks from these results as if the content of the normal serum in inorganic salts corresponded exactly to the combining power of its protein constituents, thus indicating that these salts exist in feeble chemical combination or adsorption with the proteins.

On account of the above influence of the proteins in affecting the reactivity of the serum to 'di-methyl,' and also because the degree of reactivity to these so preponderated over the reactivity of the inorganic ions, in a second series of determinations the reactivity of the inorganic salts of the serum was determined after removal of the proteins by incineration.

This method in which only inorganic salts were present was found to give very sharp results.

FIRST METHOD

REACTIVITY OF ENTIRE SERUM

TECHNIQUE

Collection of Blood.—A finger is first cleansed with methylated ether and 5 per cent. solution of formol, and then pricked with a lancet or needle. By tying a bandage or handkerchief round the wrist the superficial veins are compressed and sufficient blood may be obtained to fill one of Wright's glass capsules.

The blood is collected in these capsules by means of gravity and capillarity, or, if necessary, by suction at the other end of the tube. The ends of the capsule are then sealed in the flame of a spirit lamp or Bunsen burner, and the tube left suspended for twenty-four hours. During this time the serum separates out and, by centrifugalization, may be obtained quite free from red cells.

Preparations of Acid Solutions and Indicator.—Normal sulphuric acid was taken as the standard acid for titration. On trial, the reactivity of normal serum to 'di-methyl' was found to be equivalent to 5.5-fold dilution of normal sulphuric acid. A series of dilutions of the normal acid was then prepared, which ranged from a 3-fold to a 8-fold dilution of acid. To every 100 c.c. of these dilutions were added eight drops of 1 per cent. alcoholic solution of 'di-methyl.' This was found to be the least amount of indicator required to give the necessary colour change clearly.

As mentioned above, within certain limits, the degree of alkalinity obtained varies with the amount of indicator used. For this reason the same amount of indicator was always added to the acid.

When required for use a small quantity of mixed acid and indicator was poured into a watch glass.

Titration of Serum against the Standard Acid Solutions.—The top of the glass capsule may be easily broken off by first making a nick with a file. A capillary pipette is inserted into the serum and the latter allowed to flow into the tube for a distance of 2 cm. The serum is now allowed to run up the capillary tube for a short distance in order to introduce an air bubble, and a mark is then made at the top of the column of serum with a blue pencil. The end of the pipette is now inserted into the solution of acid against which it is to be titrated, and the acid allowed to run in until the lower end of the serum column is level with the blue mark. In this way an equal quantity of serum and of an acid of known strength is obtained. The contents of the capillary tube are blown out on to a clean white slab and thoroughly mixed with the end of the pipette and aspirated in and out of the capillary tube several times. If the red colour of the acid is not turned yellow a fresh titration is carried out with a weaker acid solution until all red disappears. The first dilution which causes a yellow colour is taken as the index of reactivity. Similarly, if at the first trial a yellow colour is produced, a stronger acid is taken until an orange red is given. The dilution which gives the last yellow colour is the index.

Preparation of Alkaline Solutions and Indicator.—For estimating the reactivity of the serum to acid in presence of phenol-phthalëin, dilutions of normal sodium hydroxide solution were used. Solutions were prepared ranging from 20-fold to 40-fold dilution.

On adding phenol-phthalëin to these alkaline solutions it was found that the colour faded in the course of a day or two. On this account when required for use 1 c.c. of the particular solution was put in a watch-glass, and a drop of 1 per cent. alcoholic solution of phenol-phthalëin added. The rest of the technique is as was described for titration against a standard acid solution.

If the pink colour given by phenol-phthalëin with the alkali does not disappear, a weaker alkaline solution is taken, and the solution at which the pink first disappears is regarded as the degree of reactivity. If at the first trial all colour disappears a stronger solution is taken, until a solution is reached which gives a faint pink colour; the solution before this is regarded as the index.

It may be here mentioned that, owing to the alkali in glass, all capsules and capillary tubes were filled with strong hydrochloric acid, and washed through several times with distilled water, and finally dried in the oven at 120°C. in order to drive off any residual acid, after this treatment distilled water left in tubes for twenty-four hours gave no acid or alkaline reaction.

CLINICAL RESULTS WITH FIRST METHOD

ENTIRE SERUM

For obtaining the normal reactivity, samples of blood were taken from the workers in the Thompson Yates and Johnston Laboratories of the University of Liverpool. Care was taken to avoid the times during which the reactivity is said to vary, viz., after food and after severe physical exertion. Blood was always taken from these cases at a stated time—12 noon—so as to avoid the effects of breakfast on the alkalinity.

In the case of the cancer patients and the disease controls, the blood was taken in the morning between 9.30 and 10 a.m.

TABLE I

REACTIVITY OF NORMAL BLOOD SERUM

No. of Case	Sex	Age	Alkalinity to di-methyl expressed as a fraction of normal	Acidity to phenol-phthalein expressed as fraction of normal
I	M	28	0.182 normal	0.033 normal
II	M	25	0.166 „	0.036 „
III	M	38	0.166 „	0.028 „
IV	M	23	0.182 „	0.033 „
V	M	30	0.182 „	0.033 „
VI	F	25	0.182 „	0.030 „
Average			0.176	0.032

TABLE II
REACTIVITY OF BLOOD SERUM IN MALIGNANT DISEASE

No. of case	Sex	Age	Disease and region	Alkalinity to dimethyl expressed as a fraction of normal	Acidity to phenolphthalein expressed as a fraction of normal
I	M	65	Epithelioma of mouth	0.166 normal	0.028 normal
II	M	65	Carcinoma of stomach	0.182 "	0.033 "
III	M	55	Epithelioma of cheek	0.200 "	0.034 "
IV	M	64	Sarcoma of neck	0.250 "	0.033 "
V	M	74	Carcinoma of tonsil	0.200 "	0.028 "
VI	M	59	Epithelioma of hand	0.200 "	0.034 "
VII	M	28	Carcinoma of stomach	0.182 "	0.030 "
VIII	M	37	Carcinoma of stomach	0.250 "	0.036 "
IX	M	52	Epithelioma of cheek	0.222 "	0.031 "
X	M	49	Carcinoma of stomach	0.200 "	0.028 "
XI	M	31	Carcinoma of rectum	0.200 "	0.033 "
XII	F	65	Carcinoma of liver	0.250 "	0.036 "
XIII	F	32	Carcinoma of rectum	0.222 "	0.033 "
XIV	F	59	Carcinoma of breast	0.250 "	0.033 "
XV	F	85	Epithelioma of hand	0.200 "	0.031 "
XVI	F	69	Epithelioma of cheek	0.222 "	0.040 "
XVII	F	67	Recurrent carcinoma of breast	0.200 "	0.030 "
XVIII	F	47	Carcinoma of colon (colotomy)	0.182 "	0.034 "
XIX	F	48	Carcinoma of colon (colotomy)	0.182 "	0.040 "
XX	F	10	Sarcoma of leg	0.200 "	0.033 "
XXI	F	52	Epithelioma of leg	0.222 "	0.033 "
XXII	F	45	Carcinoma of breast	0.200 "	0.033 "
XXIII	F	29	Carcinoma of uterus	0.200 "	0.030 "
XXIV	F	54	Carcinoma of stomach	0.222 "	0.034 "
XXV	F	41	Carcinoma of uterus	0.222 "	0.028 "
XXVI	F	27	Sarcoma of neck	0.200 "	0.033 "
Average				0.208	0.031

TABLE III
REACTIVITY OF BLOOD SERUM IN OTHER HOSPITAL CASES
(NON-MALIGNANT)

No. of case	Sex	Age	Disease and region	Alkalinity to di-methyl expressed as a fraction of normal	Acidity to phenolphthalein expressed as a fraction of normal
I	M	65	Chronic bronchitis	0·182 normal	0·040 normal
II	M	65	Chronic bronchitis	0·200 "	0·034 "
III	M	35	Paralysis of legs	0·182 "	0·030 "
IV	M	78	Chronic bronchitis	0·182 "	0·028 "
V	M	44	Old fracture of thigh	0·222 "	0·033 "
VI	M	49	Ulcer of leg	0·200 "	0·037 "
VII	M	45	Sprained shoulder	0·182 "	0·048 "
VIII	M	56	Wound of scalp	0·166 "	0·040 "
IX	M	45	Old hip disease	0·166 "	0·036 "
X	M	57	Ulcer of leg	0·182 "	0·033 "
XI	M	41	Varicose veins of leg	0·200 "	0·033 "
XII	M	43	Abscess of neck	0·166 "	0·022 "
XIII	M	31	Tertiary syphilis	0·182 "	0·033 "
XIV	M	49	Sprained arm	0·166 "	0·037 "
XV	M	45	Ulcers of leg	0·166 "	0·036 "
XVI	M	38	Tubercular knee	0·166 "	0·028 "
XVII	M	34	Tubercular thigh	0·182 "	0·033 "
XVIII	M	19	Tubercular knee	0·182 "	0·037 "
XIX	M	47	Tubercular hip	0·200 "	0·025 "
XX	M	23	Tubercular pelvis	0·182 "	0·033 "
XXI	M	57	Diffuse scaly eczema	0·153 "	0·025 "
XXII	F	19	Moist eczema of face and arms	0·182 "	0·027 "
Average				0·181	0·033

TABLE IV

EFFECT OF AN INCREASED AMOUNT OF INDICATOR ON REACTIVITY
OF BLOOD SERUM IN MALIGNANT DISEASE

No. of case	Sex	Age	Disease and region	Alkalinity to di-methyl expressed as a fraction of normal
I	M	79	Rodent ulcer on cheek	0.166 normal
II	M	62	Epithelioma of mouth	0.166 "
III	M	37	Glands in neck secondary to epithelioma of lip	0.166 "
IV	M	38	Epithelioma of lip	0.166 "
V	M	69	Epithelioma of lip	0.153 "
VI	M	61	Sarcoma of jaw	0.166 "
VII	F	36	Carcinoma of uterus	0.166 "
VIII	F	78	Rodent ulcer on nose	0.153 "
IX	F	71	Carcinoma of breast	0.166 "
X	F	40	Carcinoma of uterus	0.153 "
XI	F	67	Sarcoma of neck	0.153 "
XII	F	75	Epithelioma of lip	0.153 "
XIII	F	64	Carcinoma of breast	0.166 "
XIV	F	48	Carcinoma of breast	0.166 "

NOTE.—Normal serum with the same amount of indicator reacted to 0.153 to 0.143 normal.
The average for the above fourteen cases is 0.161

CONSIDERATION OF RESULTS OF FIRST METHOD

On examining these tables it is evident that, as regards acidity to phenol-phthaleïn, no definite conclusions can be drawn.

It is true that some cases show an increased acidity with a decreased alkalinity, but on the whole no definite relation can be established. On considering the average of the values obtained, it is seen that—

6 healthy cases	show an average of	0.032 normal
26 cancer cases	" "	0.031 "
22 cases of other diseases	" "	0.033 "

The alkalinity to 'di-methyl' on the other hand shows some striking results.

The upper limit of alkalinity for healthy subjects is 0.182 normal

Now of 26 cancer cases, 21 are increased, while of 22 control cases of other diseases, which were nearly all at or near the cancer age, only five are above the normal alkalinity.

Average of 6 healthy cases	...	0.176 normal
" 26 cancer cases	...	0.208 "
" 22 cases of other diseases		0.181 "

SECOND METHOD

REACTIVITY OF INORGANIC SALTS OF SERUM

TECHNIQUE

Collection of Blood.—The blood is collected in the same manner as in the first method. The capsules are left suspended for twenty-four hours and then centrifugalised.

Standard Acid Solutions and Indicator.—Dilutions of normal sulphuric acid are prepared commencing with a 28-fold dilution, and advancing by 0·5-fold dilutions to 40-fold.

The indicator used is 'di-methyl.' When an acid solution is required for use, 0·5 c.c. is put in a watch-glass and to it is added the 'di-methyl.' The amount of indicator used in any series of observations should always be the same. In the present investigation the following plan of ensuring this was adopted :—A piece of thick glass tubing of fine calibre, about 20 centimetres in length, was drawn out at one end to a fine point. The rest of the tube was covered with paraffin wax and twenty·15 centimetre divisions etched on. The tube was now calibrated by means of mercury, and each centimetre length was found to have a capacity equal to 0·00475 gram of water. Hence each division on the tube corresponded to a capacity of 0·00288 c.c. The tube was filled with di-methyl, and to each·5 c.c. of acid solution was added the contents of three of these divisions. This is a simple way of ensuring that the amounts of acid solution and indicator always bear the same ratio to each other.

Incineration.—The serum is allowed to run up a capillary tube for a distance of 6 or 7 centimetres and a mark is made with a blue pencil opposite the top of the column of serum. The serum is now blown out on to a small platinum dish, which is then transferred to a drying oven in order to ensure the complete evaporation of moisture without any spurting. When perfectly dry the platinum dish is placed over a Bunsen flame and all organic matter completely incinerated.

The capillary tube is washed out two or three times with distilled water and the washings transferred to the platinum dish, to be in turn dried and incinerated. In this way all residual serum is got rid of from the tube.

The platinum dishes used are about the size of a penny piece, and in the centre a well is made about the diameter of an ordinary lead pencil and 4 m.m. in depth.

In this well serum and washings are placed, and in it all titrations against the standard acid solutions are made. A simple but quite effective drying oven can be made out of any tin with a hinged lid, such as some tobacco tins, by inserting a shelf of wire gauze.

Titration against Standard Acid Solutions.—The acid solution and the indicator are placed in a watch glass, as mentioned above. Another watch glass containing some of the acid solution, but no indicator, is also required. This is for washing out the capillary tube which contained the serum, after it has been washed out with distilled water. The capillary tube is now plunged into the mixed acid and indicator solution

which is allowed to run up to the blue mark. In this way equal amounts of serum and acid solutions are used. The acid is now blown out into the well of the platinum dish which contains the dried inorganic salts of the serum. Titration is effected by means of a small glass rod, through which the colour changes can be very clearly seen.

If after titrating for two or three minutes the pink colour still persists, a fresh titration with a weaker acid solution is undertaken until the colour disappears. The point at which the colour first completely disappears is taken as the neutral point. Similarly if the colour goes at the first trial a stronger acid is taken, until the colour faintly persists. The solution before this point is regarded as the index.

After each titration the platinum dish and the glass rod are thoroughly washed with distilled water.

ADVANTAGES OF THE METHOD.

1. Owing to the small amount of apparatus necessary, and the small quantity of blood required, the method may be used clinically.

2. Although serum was used in the research described below in order to compare the results with those obtained by the first method, nevertheless, the total blood may be used as all organic matter is burnt off. This is a great advantage as in all other methods in which the total blood is used the results are obscured by the colouring matter and the great dilution necessary to overcome it. Moreover, as Loewy has pointed out, with these methods, unless the blood be titrated slowly and at the body temperature, all the corpuscles are not broken down, and the total alkalinity is therefore not detected, and that probably, owing to the neglect of this precaution, previous results have given too low values.

3. The colour change is very sharp and clearly defined. The delicacy of the method is proved by the fact that the colour often goes on using a 0.5-fold weaker acid, which contains an amount of acid very little less than the solution which was too strong,

4. Although only small quantities of serum are used, the accuracy of results, judged by controls obtained by using large amounts, is remarkable.

It is important that all glass capsules and capillary tubes should be made free from excess of alkali by the means described under the first method.

CLINICAL RESULTS OF SECOND METHOD

INORGANIC SALTS OF SERUM

The aims of the research, for which this method was used, were—

1. To establish the normal reactivity of the inorganic salts in the serum, in both men and women.

2. To find out in what ways, if any, these salts varied from the normal in malignant disease.

The blood for these observations in all cases was taken at least three hours after breakfast so as to exclude the possible influence of digestion. None of the subjects had indulged in severe physical exertion on that day.

Some of the cases of malignant diseases had been operated on when their blood was removed.

TABLE V

BASIC REACTIVITY OF INORGANIC SALTS OF NORMAL BLOOD SERUM

No. of case	Sex	MALES	
		Age	Alkalinity to di-methyl expressed as a fraction of normal
I	M	29	0.0312 normal
II	M	26	0.0312 "
III	M	23	0.0307 "
IV	M	26	0.0312 "
V	M	24	0.0312 "
VI	M	25	0.0292 "
VII	M	28	0.0307 "
VIII	M	36	0.0307 "
IX	M	25	0.0312 "
X	M	25	0.0303 "
XI	M	39	0.0307 "
XII	M	25	0.0307 "
XIII	M	27	0.0307 "
XIV	M	23	0.0307 "
XV	M	32	0.0303 "
XVI	M	34	0.0312 "
XVII	M	29	0.0292 "
XVIII	M	23	0.0312 "
XIX	M	22	0.0303 "
XX	M	22	0.0294 "
XXI	M	26	0.0303 "
XXII	M	22	0.0312 "

TABLE VI

BASIC REACTIVITY OF INORGANIC SALTS OF NORMAL BLOOD SERUM

FEMALES			
No. of case	Sex	Age	Alkalinity to di-methyl expressed as a fraction of normal
I	F	...	0.0303 normal
II	F	...	0.0292 "
III	F	...	0.0322 "
IV	F	...	0.0307 "
V	F	...	0.0303 "
VI	F	...	0.0322 "
VII	F	...	0.0292 "
VIII	F	...	0.0307 "
IX	F	...	0.0312 "
X	F	...	0.0307 "
XI	F	...	0.0307 "
XII	F	...	0.0292 "
XIII	F	...	0.0303 "
XIV	F	...	0.0312 "
XV	F	...	0.0307 "
XVI	F	...	0.0307 "

TABLE VII
BASIC REACTIVITY OF INORGANIC SALTS OF BLOOD SERUM
IN MALIGNANT DISEASE

MALES					Alkalinity to di-methyl expressed as a fraction of normal
No. of case	Sex	Age	Disease and region		
I	M	63	Epithelioma of tongue		0·0322 normal
II	M	48	"	"	0·0327 "
III	M	63	"	"	0·0317 "
IV	M	70	"	"	0·0322 "
V	M	63	"	"	0·0312 "
VI	M	38	"	" (Recurrent)	0·0317 "
VII	M	54	"	" "	0·0312 "
VIII	M	43	"	" "	0·0312 "
IX	M	53	"	" "	0·0307 "
(Five days after operation).					
X	M	25	Carcinoma of Rectum		0·0327 "
XI	M	61	"	"	0·0317 "
XII	M	38	"	"	0·0317 "
XIII	M	51	"	"	0·0317 "
XIV	M	59	"	"	0·0303 "
XV	M	45	"	"	0·0327 "
(Fourteen days after operation)					
XVI	M	53	Carcinoma of Rectum		0·0307 "
(16 days after operation)					
XVII	M	58	Rodent ulcer of lips		0·0333 "
XVIII	M	75	"	eyelid	0·0322 "
XIX	M	79	"	cheek	0·0317 "
XX	M	48	Epithelioma of penis		0·0322 "
XXI	M	47	"	"	0·0307 "
(Nine days after operation)					
XXII	M	64	Carcinoma of Oesophagus		0·0327 "
XXIII	M	44	"	Colon	0·0327 "
XXIV	M	36	Sarcoma of jaw		0·0312 "
XXV	M	54	"	neck	0·0322 "
XXVI	M	18	"	testicle	0·0322 "
XXVII	M	57	"	neck	0·0333 "

TABLE VIII

BASIC REACTIVITY OF INORGANIC SALTS OF THE BLOOD SERUM
IN MALIGNANT DISEASE

FEMALES					Alkalinity to di-methyl expressed as a fraction of normal
No. of case	Sex	Age	Disease and region		
I	F	50	Carcinoma of breast	0.0317 normal	
II	F	52	" "	0.0333 "	
III	F	56	" " (Recurrent)	0.0327 "	
IV	F	57	" " "	0.0307 "	
V	F	52	" "	0.0322 "	
VI	F	56	" "	0.0307 "	
VII	F	37	" "	0.0327 "	
VIII	F	58	" "	0.0312 "	
IX	F	38	" "	0.0303 "	
X	F	50	" "	0.0312 "	
XI	F	39	" "	0.0333 "	
XII	F	47	" "	0.0312 "	
XIII	F	56	" "	0.0302 "	
XIV	F	37	" "	0.0327 "	
			(7 days after operation)		
XV	F	53	Carcinoma of breast	0.0303 "	
			(14 days after operation)		
XVI	F	49	Carcinoma of rectum	0.0312 "	
XVII	F	29	" "	0.0317 "	
XVIII	F	37	" "	0.0312 "	
XIX	F	64	Malignant glands in axilla	0.0307 "	
			(2 days after operation)		
XX	F	48	Carcinoma of thyroid gland	0.0322 "	
XXI	F	45	Epithelioma of tongue	0.0322 "	
			(14 days after operation)		
XXII	F	62	Rodent ulcer of eyelid	0.0322 "	
XXIII	F	38	Sarcoma of breast	0.0312 "	
XXIV	F	8	" skin of arm	0.0322 "	

TABLE IX
BASIC REACTIVITY OF INORGANIC SALTS OF BLOOD SERUM IN
OTHER DISEASES

No. of case	Sex	Age	Disease and region	Alkalinity to di-methyl expressed as a fraction of normal
I	M	34	Septic anaemia	0.0277 normal
II	M	32	Tubercular hip	0.0292 "
III	M	28	" knee	0.0303 "
IV	M	25	" shoulder	0.0312 "
V	M	12	Diabetes	0.0285 "
VI	M	33	"	0.0312 "
VII	M	38	"	0.0303 "
VIII	M	21	"	0.0307 "
IX	M	39	"	0.0289 "
X	F	43	"	0.0307 "
XI	F	30	Eclampsia	0.0303 "
XII	F	62	Tubercular breast	0.0292 "
XIII	F	16	Adenoma of Thyroid gland	0.0292 "
XIV	F	28	Mammary abscess	0.0307 "
XV	F	18	Psoriasis	0.0307 "

TABLE X. AVERAGES

No. of cases	Sex	Condition of health	Average alkalinity to di-methyl expressed as a fraction of normal
22	M	Healthy	0.0304 normal
16	F	"	0.0306 "
38	M + F	"	0.0305 "
27	M	Cancer	0.0318 "
24	F	"	0.0316 "
51	M + F	"	0.0317 "
15	M + F	Diseases other than cancer	0.0297 "

SUMMARY AND CONCLUSIONS

1. Although the results of physical methods show that the ratio of the concentrations of hydrogen and hydroxyl ions in the plasma is a comparatively low one, thus indicating an approximation to neutrality ; yet the cells are so extremely sensitive, in their metabolism, growth, and nuclear division, to small variations in this ratio, that it becomes essential to study the effects of small variations in the ratio around the neutral points upon the cells.

2. Such small variations cannot at present be followed by any known physical method, and the only approximate method available is that of determining the *reactivity* of the plasma or serum to acids and alkalies by means of indicators.

3. Coloured indicators for acids and alkalies do not show the neutral point of equality in concentration of hydrogen and hydroxyl ions, but a point at which the ratio of the concentrations of these two ions has a certain comparatively *low* value. The value of the ratio at the neutral colour point is dependent upon the ratio of dissociation and association of the indicator, and this in turn upon the nature of the indicator, and secondarily upon the concentration of the indicator. Hence one indicator changes for low hydrogen ionic concentration (such as phenol-phthalëin), and another for low hydroxyl ionic concentration (such as di-methyl-amido-azo-benzol). The ratio of the concentrations in the case of serum lies at a low intermediate value, so that it gives the acid colour with phenol-phthalëin, and the alkali colour with 'di-methyl.'

4. The resistance of the serum to rapid displacement of the ratio in the two ions when acid or alkali is added may be termed the *reactivity* of the serum. The amount of alkaline reactivity may be determined by titration with acid in presence of a strong indicator such as 'di-methyl' or methyl-orange, which changes with a concentration of hydrogen ions somewhat higher than that of distilled water⁽¹⁾; and the

1. That this range in concentration is not a pronouncedly acid one is shown by the fact that about 1 c.c. of decinormal hydrochloric acid in 1 litre of distilled water (i.e. a 1/100 normal solution) give as distinctly acid reaction with 'di-methyl' or methyl orange.

amount of acidic reactivity by similar titrations with alkali in presence of a weak indicator which changes with a concentration in hydroxyl ions somewhat higher than in distilled water (*e.g.*, phenol-phthalëin).

In all cases the indicator used for determining the reactivity should be stated, *e.g.*, the alkaline reactivity of the entire serum to di-methyl is 0.166 to 0.182 normal, and its acidic reactivity to phenol-phthalëin is 0.028 to 0.036 normal.

5. The long range in reactivity possessed by the serum (and also by the plasma and other body and tissue fluids) is due (*a*) to the inorganic salts such as bi-carbonates and primary and secondary phosphates of the alkali metals which it contains, and (*b*) to the proteins present which are capable of functioning as feebly dissociated acids or bases, and so restraining any great movement of the ionic ratio in either direction outside of physiological limits.

This range of reactivity is essential to the continuance of life, because of the extreme sensitivity of the living cell to variations in the ratio of concentration of the two ions.

6. The susceptibility of the living cell to increase in concentration of either hydrogen or hydroxyl ions is due to the fact that the protein of the cell possesses strong affinity for both these ions forming feebly disassociated salts with them. So that in the presence of higher concentration than the normal of either ion the protein becomes fixed, and no longer possesses that liability of composition and affinity for attaching organic nutrient substances which is essential to its metabolism.¹

Just as acids and alkalies affect the association or dissociation of coloured indicators and alter their colour by means of the changes which the acids or alkalies cause in concentrations of the two ions, so do acids and alkalies affect living cells and the proteins of the plasma and tissue fluids, and the picture given by the coloured indicators may be regarded as a model illustrating what occurs in the action of acids or alkalies upon living cells.

1. The action of all anaesthetics is similar in character. See Moore and Roaf, *Proc. Roy. Soc.*, Vol. 73, 1904, p. 382; *Ibid.*, B, Vol. 77, 1905, p. 86.

7. The high value for the alkaline reactivity of the proteins of the serum to 'di-methyl' found above (0.15 normal) is of interest, because of its close coincidence with the molecular concentration of the *total* inorganic salts of the serum, as shown by depression of freezing point or incineration methods (0.14 normal), indicating that there probably exists an adsorption or combination between the proteids and the total inorganic salts of the plasma which regulates the total amount of such salts in the plasma.

8. Values have been obtained for the acidic reactivity of the entire serum to phenol-phthalëin, and the basic reactivity to 'di-methyl' in (a) normal individuals, (b) cases of malignant disease, and (c) cases of disease or debility from other causes.

No constant variations have been found in the index of acidic reactivity, the averages running:—0.032 normal; 0.031 cancer; 0.033 other cases. A definite variation of basic reactivity has been found in the direction of increased alkalinity in the malignant cases, the averages running:—0.176 normal; 0.208 cancer; 0.181 other cases.

9. Values have also been obtained for the basic reactivity to 'di-methyl' of the inorganic salts of the serum after removal of the proteins by incineration. Such determinations have been carried out in normal individuals of both sexes, in cases of malignancy in both sexes, and in cases of non-malignant disease or debility.

The results show (a) that there is no appreciable variation with sex either in normal individuals, nor as between the two sexes, in malignancy, and (b) that there is a small but distinct increase in basic reactivity in the malignant cases, which is not shown by the non-malignant cases. The averages run:—Health, 0.0304 (males), 0.0306 (females); malignant cases, 0.0318 (males), 0.0316 (females); non-malignant cases, 0.0297 (males and females).

10. The difference in direction of increased alkalinity in cancer is less after removal of the protein than before its removal, indicating the possibility of the proteins in cancer possessing a higher basic reactivity than normal.

11. Although the differences in basic reactivity between the serum of malignancy and that of normal individuals are small (about 16 per cent. in the whole serum and 4 per cent. in the inorganic salts) yet they are quite definite, and entirely outside the region of experimental error of the methods employed.

The point of neutral colour is particularly sharp in the determinations of the inorganic reactivity, where we feel quite certain we can depend upon the accuracy of each reading. Further, the possibility of error from the minute quantities taken was considered, and duplicates done in the sera of animals, by the clinical method, and by controls on the large scale in platinum vessels. As a result, a very close accord was found between the two sets of results.

With the whole serum the end point is not so sharp on account of the presence of the proteins, but duplicate determinations were always carried out, and the experimental errors possible lie well within the larger difference of 16 per cent. in the two averages.

Also the series of cases observed are of considerable length, and the large preponderance of the malignant cases lying above normal is obvious from the tables.

12. With regard to the important point as to whether this increased basic reactivity in malignancy is to be regarded as a cause or an effect of the disease it may be pointed out (*a*) that the increased basicity is present in early cases where there could not probably be any large amount of altered material coming from the seat of the growth; (*b*) that the increased basicity is present after removal of the growth; and (*c*) the important fact that there is a general concurrence in the results of earlier observers, that there is an increase in alkalinity (reactivity) of the serum in old age when the organism is liable to the onset of malignancy.