Colorimetric Estimation of Phosphorus

To determine the rate of colour formation and the optimum time for taking readings in the Fiske and Subbarow method of estimating phosphorus microchemically, we have used a simple type of photoelectric colorimeter (to be described in detail elsewhere) which allows the chemical reaction to be followed continuously until a state of equilibrium is reached. The method has been developed for the purpose of obtaining more accurate figures for the phosphorus content of the whole blood and its separate fractions (plasma, serum and red cells) than those hitherto obtained by other (visual) methods.

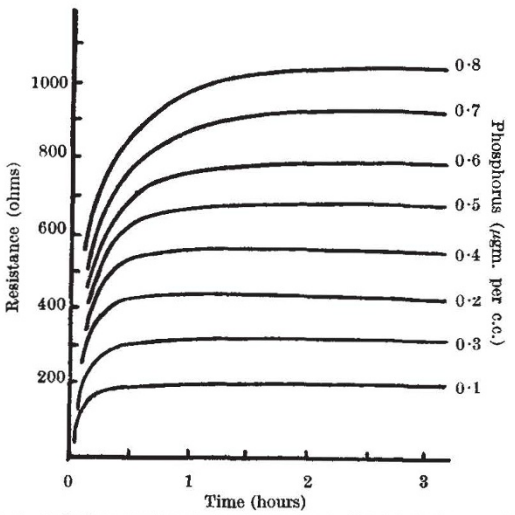

FIG. 1. Relation of colour intensity to time. Curves for the smallest amounts of phosphorus measured have been omitted; in the interests of clearness, the steep ascents in the earlier stages of colour formation have also been omitted.

Our apparatus enables us to detect 10^{-9} gm. of phosphorus per c.c. of solution, and to measure accurately changes of less than 10^{-7} gm. The reagent used, which differs slightly from that given by Fiske and Subbarow, consists of 0·1 gm. of 1 : 2 : 4 aminonaphthol-sulphonic acid, 5·48 gm. of sodium metabisulphite, 1·2 gm. of crystalline sodium sulphite dissolved in distilled water to make 50 c.c. of the solution. When the component substances are in these proportions, the acid remains in solution unless the temperature changes markedly. A beam of approximately monochromatic light which, passing through a liquid filter containing the solution to be examined, falls on to a photo-electric cell and so gives rise to a photo-electric current, is balanced against a current generated by a beam from the same source, which has, however, passed through a glass vessel containing only distilled water. After the addition of 2 c.c. of an ammonium molybdate and sulphuric acid solution and then of 1 c.c. of the modified Fiske and Subbarow reagent to the test solution, the characteristic blue colour that forms may be followed by means of a variable resistance which is manipulated so that the spot of light of a mirror galvanometer (of low resistance) remains undeflected.

By measuring solutions containing known amounts of phosphorus (from 0·03 to 1 microgram per c.c.) we calibrated the instrument to express intensity of colour in terms of the balancing resistance; it was found that, roughly, a difference of 0·05 microgram of phosphorus per c.c. produced a colour which caused a change of resistance of 50 ohms, a difference of 1 ohm producing an easily perceptible deflection even with an ordinary laboratory galvanometer. The calibration curve is very nearly linear. When the intensity of colour, expressed in ohms, is plotted against time, curves of the type shown in Fig. 1 are obtained.

These curves show that the colour begins to form immediately after the reagents have been added and increases rapidly in intensity until a comparatively steady state is reached: actually, the colour continues to deepen for many hours. When the quantity of phosphorus present is very small, this approximately steady value is attained in about 20 minutes, but when the amounts are of the order of 0·5 μ gm. per c.c., the colour should not be read until two hours have elapsed since the reagents were added. The curves shown in Fig. 1 are drawn from readings taken at intervals of 2½ minutes in the early stages, then 5 minutes, later 15 minutes and hourly. It also follows from these curves that, when the colours are read in the ordinary ocular colorimeters, the varying rates of development of colour in the standard and the unknown will escape notice owing to the relative insensitiveness of the method.

A merit of the present apparatus is that once the calibration curve for standard amounts of phosphorus has been determined, it is no longer necessary to prepare a standard for comparison with the unknown solution, since the liquid filter used to obtain an electrical balance need contain only distilled water. The advantage of being able to measure accurately the successive changes of colour in the solution is obvious.

The same method is now being applied to other standard colorimetric tests, and it appears probable that the interval of time within which the respective colours are to be read will be found, as in the present case, to be greater than that usually allowed, since more accurate results will be obtained when the measurements are made on the nearly horizontal branches of the resistance curves. In some tests, such as that for cholesterol, it will be possible to determine the optimum time for reading the colour before it begins to fade.

Under the auspices of the Cancer Research Committee of the University of Sydney, we are using the above method to measure the amounts of certain constituent substances in pathological human blood.

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Surface Properties of Non-Aqueous Solutions

A CLOSE study of the surface properties of capillary-active solutions of compounds forming homologous series has shown that the surface activity increases regularly with the molecular weight (Traube's rule). Only aqueous solutions have, however, been thoroughly investigated, and little is known of non-aqueous systems. Since organic solvents have much lower surface tensions than water, the changes produced by solutes will doubtless be much smaller, but the following results show that in certain solvents interesting effects of a similar nature can be observed.

I have determined the surface tension of solutions of different normal alcohols in aniline by means of a Traube stalagmometer. Aniline was chosen as solvent because of its comparatively high surface tension ($\varsigma_{30^\circ} = 42{\cdot}9$ dynes/cm.). Although the lowering of the surface tension ($\Delta\varsigma$) is much smaller than in aqueous solutions, it was clearly established that, as

in water, $\Delta\varsigma$ is the greater the higher the concentration (c) of the alcohol and that for isomolecular solutions of different alcohols $\Delta\varsigma$ is the greater the higher the molecular weight. The surface tension-concentration curves were of the same type as generally encountered in aqueous solutions, that is, ς falls most rapidly at small concentrations. For dilute solutions (c less than 0·1 mol./lit.) $\Delta\varsigma/c = F$ can be regarded as constant and the following table gives the F-values for the different alcohols :

Alcohol	F
Ethyl	(1·6)
Propyl	3·4
Butyl	6·5
Amyl	9·6
Hexyl	12·8
Octyl	18·8
Decyl	25·2
Dodecyl	31·0

While in aqueous solutions F progresses geometrically as we ascend the homologous series, we find here a regularity of a less pronounced character, namely, from propylalcohol onwards the F-values form an arithmetical progression as do the molecular weights. Thus while in aqueous solutions F_{n+1}/F_n is a constant (generally 3), we find here $F_{n+1} - F_n =$ constant $= 3$.

The fact that F_{n+1}/F_n approaches the value 1 as we move up the series indicates that the influence of the additional CH_2-group upon the surface energy becomes the smaller as the hydrocarbon-chain becomes longer.

It remains to be seen whether in other cases where the solutes have much smaller surface tensions than the solvent similar relationships will be found.

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Rate of Absorption of Oxygen by Sodium Sulphite Solution

DURING the course of some measurements on the rate of absorption of gaseous oxygen by aqueous sodium sulphite solution, it was found that, under certain conditions, stirring the liquid beneath the surface without in any way agitating the latter *decreased* the rate of absorption below that of the unstirred liquid.

The apparatus used consisted essentially of a glass tube into the bottom of which a glass rod was sealed to act as a pivot for a glass stirrer below the liquid surface. This stirrer was hollow and contained iron wire so that it could be moved, by means of a magnetic field rotated externally, without breaking the liquid surface at any point. The other end of the tube was connected to a manometer. This portion was constructed in triplicate to ensure adequate control, and provision was made whereby the whole apparatus could be exhausted and the gas to be used admitted as required, each tube then being connected separately to its particular manometer.

Two tubes of approximately normal sodium sulphite solution were compared; each of these contained similar stirrers but only one of them was stirred (care being taken not to agitate the liquid-gas surface). After the initial rapid absorption, when the rate had become constant and after applying corrections, it was found that the rate of gas absorption by the solution the bulk of which was stirred was in general 48 per cent *less* than the rate at which the gas was absorbed by the unstirred liquid.

A systematic study of this phenomenon is being undertaken, and it is hoped to publish some of the results of the investigation shortly.

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Enolization of Oxycholesterilene

$\Delta_{5:6}$-UNSATURATED sterols may be converted to the 7-dehydro compounds by the method of Windaus, Lettré and Schenck[1]. An alternative route, which is being examined in these laboratories, lies in the enolization of 7-ketocholesterol (I).

Acetylation of (I) resulted in the removal of the C_3-hydroxyl, oxycholesterilene and the acetate of oxycholesterilene being obtained. The latter compound (II) had m.p. 90°–92°, $[\alpha]_D$ − 222°, $[\alpha]_{5461}$ − 283° (found : C, 82·0; H, 10·9. Calc. for $C_{29}H_{44}O_2$: C, 82·1; H, 10·4 per cent). Unless a shifting of the double bonds has taken place, this compound (II) will have the structure shown in

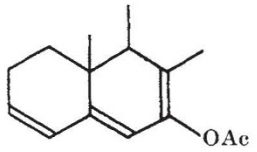

FIG. 1.

Fig. 1. Its ultra-violet absorption spectrum has been examined by Dr. R. K. Callow, who reports that it is consistent with this formula. In view of the mobility of the ergosterol ring system, however, the product m.p. 90°–92° may be contaminated with an isomeride.

This point is at present under investigation. Methods for the preparation of $\Delta_{5:6:7:8}$-cholestadiene-3 : 7-diolacetate are being examined and the results will shortly be published elsewhere.

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[1] *Ann.*, **520**, 98 (1935).

Bilateral Gynandromorphism in Feathers

LILLIE and Juhn[1] suggested that the reactivity to œstrone sometimes manifested only on one side of a feather depends upon a low growth-rate of the tissue at the time, which rate they considered may be different on the two sides. These original views were immediately criticized by other workers[2,3]. They seem to have been greatly modified in exhaustive papers by Fraps and Juhn[4]; but it does not appear that the senior author has abandoned them.

Greenwood and Blyth[5] have shown that asymmetrically coloured feathers may be produced in the Brown Leghorn capon by the intra-dermal injection of small doses of œstrone. I have sectioned the bases of six asymmetrically marked feathers obtained in this way in which the rachis was substantially straight and the barbs on each side of a similar length. I have counted in a section the barbs cut on each side and compared the lengths of the two halves of the collar. The side of the feather which had reacted most to œstrone corresponded with the larger side of the germ and had therefore on the concrescence theory presumably grown more slowly (as required by the theory of Lillie and Juhn) in two