after specific treatment. Boen (1957) has studied similar changes in mouth washings from cases of pernicious anaemia. A small series of patients and normal controls was studied, and the mean long diameter of the squamous-cell nucleus in certain of his cases was found to be abnormally large. Boddington (1959) has confirmed that enlargement of the nuclei from desquamated oral epithelial cells occurs in megaloblastic anaemia. More particularly this enlargement is related to epithelial atrophy of the tongue. Such a relation was not noted in this work (Farrant, 1958b), but the recognition of minor degrees of atrophy is difficult. In addition the epithelia studied may not be comparable.

In the present study variability of nuclear size in buccal epithelium has been found to be increased, and this is at least as characteristic a change as that of greater mean size in pernicious anaemia. In his study of the diameter of red blood cells in pernicious anaemia, Price-Jones (1933) found a correlation between variability of red-cell size and the number of red cells, and a less strict correlation between variability of size and haemoglobin level. He also noted that the mean size of the red cells, as opposed to their variability, bore no relation to the degree of anaemia. The present work has shown that the size of squamous-cell nuclei, too, is independent of the degree of anaemia.

The change to normal size and shape of the nuclei was observed to occupy only a few days once treatment in pernicious anaemia was started. Not infrequently a longer time may be needed for a smooth tongue to become normal. If enlargement of squamous-cell nuclei is a consequence of failure of normal nuclear division, and if changes in the basal layer of the tongue are similar in nature to those found in buccal epithelium, then impairment of growth of cells at the base of the filiform papillae might occur. This would allow desquamation of cornified cells from the tip of the papilla to exceed regeneration from below, and papillary atrophy would result. Conversely, when treatment began and normal cell divisions started, the need for increased regeneration to exceed continuing desquamation might account for the delay in the return to normal of the lingual epithelium, as compared with the changes caused by treatment which have been demonstrated in this study. Impaired activity of the basal cell layer of the tongue has been demonstrated in the glossitis of pernicious anaemia by Taft, Hughes, and Wood (1958).

In a few patients with untreated pernicious anaemia the squamous-cell nuclei have been found to be of normal size. Vitamin-B₁₂ deficiency may not always present in the same clinical form, and very low levels of serum vitamin B₁₂ have been reported (Mollin and Ross, 1954) in a small group of patients with subacute combined degeneration of the cord in the absence of significant anaemia. Sanford (1935), Brown (1946), and Bastrup-Madsen (1954) have all described the simultaneous occurrence of oral and neurological manifestations with little or no anaemia. The patients with squamous-cell nuclei of normal size did not, however, differ from the others with regard to the incidence of glossitis or subacute combined degeneration of the cord, and a cause for the presence in them of nuclei of normal size was not discovered.

Summary

By measuring squamous-cell nuclei from the mouth, abnormalities of nuclear size, shape, and variability of size have been demonstrated in pernicious anaemia. Treatment causes these abnormalities to regress. The possible significance of these findings is discussed.

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THE E.S.R. IN A NEW DRESS

BY

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The E.S.R. is one of the oldest investigations in clinical medicine, and appears to have developed in a crude form from observations on the blood in relation to "blood-letting." The first modern investigation of the diagnostic possibilities of the test was made by the Swedish physician Fåhræus (1921, 1929), who attempted to make use of a raised E.S.R. as a diagnostic test for pregnancy. He failed in his thesis, but developed a method of E.S.R. estimation that his pupil Westergren, working with male tuberculosis cases in Stockholm, was later to improve upon and publish in his classical paper in 1921.

Other methods have since been tried, notably that of Wintrobe (1933), but for various reasons, not discussed here, they have fallen from favour, and the Westergren method and its values still form the foundations for present-day clinical thinking.

The substance of this paper arose from the gradual evolution of hybrid methods for estimating the E.S.R. (or, more exactly, the erythrocyte aggregation sedimentation rate) as a result of laboratory modifications of the classical Westergren method without any

apparent evaluation of their relative accuracy or revision of the standards used. The methods that had developed in Edinburgh were suspected of serious anomalies, and this communication represents an attempt to clarify the situation.

Practical and Theoretical Considerations

The numerical value of the E.S.R. is largely dependent on those factors which affect the red-cell surface and on the concentration and molecular shape of the associated serum proteins; hence, for example, the needle-like fibrinogen molecule will produce a higher E.S.R., weight for weight, than will the rounded molecule of albumin.

It is now generally believed that an accurate and reproducible E.S.R. based on known normal standards does, if elevated, indicate a non-specific somatic reaction to disease.

A comprehensive study of the multitude of factors that can affect the E.S.R. has been made by Nichols (1942), working with horses' blood; but for clinical purposes the main physico-chemical and other factors affecting the E.S.R. are more limited and include:

Major Factors.—(1) The dimensions of the tube used in the estimation itself. (2) The verticality of this tube. (3) The use with the blood sample of a liquid or a solid as an anticoagulant—for example, solid "sequestrene" (ethylenediaminetetra-acetic acid; E.D.T.A.), liquid citrate. (4) The technique associated with venepuncture, including trauma, contamination, and cleanliness of apparatus. (5) The total time over which the estimation is made.

Minor Factors.—(1) The time elapsing between withdrawing blood from the patient and testing it. (2) The anticoagulant chosen and the care taken in mixing in different cases. (3) The surrounding temperature in moderate climates. (4) The "reflux factor" of Alexander (1953). (5) The choice between arterial and venous blood samples and their gas content. (6) Personal factors—frustration, clumsiness, and fatigue.

It has been shown from past studies on the E.S.R. that when anticoagulated venous blood is subjected to all the above physico-chemical variants in a suitable tube it undergoes three major phases—aggregation, fall, and pile-up (Fig. 1).

FIG. 1.—Showing curve during the three phases.

The first phase, that of red-cell aggregation, should be as short as possible. The reason for this is that measurement of the E.S.R. is, for sake of convenience, taken from the setting up of the tube, and not, as strictly speaking it should be, from the time when aggregation

is complete and fall begins; and therefore phase 1, the formation of the aggregation, is included in the final result.

However, it is in the second phase that the chief interest lies, for this represents the falling of the red-cell aggregates under a steady gravitational influence which, ideally, is graphically linear. When this is so, it can reasonably be assumed that the forces involved are acting on a complete and stable state of aggregation, and the drop can then be read in one of two ways: either as time in minutes per millimetre unit of fall, or as fall in millimetres per unit time (one hour).

The third phase results from the effect on the falling red-cell column of its leading members piling up at the bottom of the tube, and this is a very real factor in rapid E.S.R.s. If, however, the tube is of reasonable length, then it is found that this phase does not affect the result over a one-hour period unless the aggregated fall is over some 30% of the tube length. This means that with a tube of 200 mm. a result of up to 70 mm. in one hour would be unaffected.

Against this background it became necessary to establish which was the most convenient method of determining the E.S.R. in a routine comprehensive haematological service.

Aims

1. To develop a method which would use sequestrene as an anticoagulant and to calibrate it against the standards of present accepted methods, and which would, in addition, conform with the following requirements: (a) It could be adapted from apparatus already in general use and ideally would employ present accepted normal standards. (b) It would be standardized on anticoagulated samples of venepuncture blood, both in technique and in equipment, and should be reproducible in any laboratory.

2. (a) To find a method of suspending a pipette so as to increase the accuracy of the E.S.R. estimation; this was essential owing to the very considerable effect any deviation from the vertical had on the reading obtained. (b) To develop a tube that would be cheaper, simpler, and less frustrating than the type in present use.

3. To compare the various methods involved in E.S.R. estimation: (a) "classical" and "modified classical Westergren"; (b) using undiluted blood with Heller's ammonium potassium (NH_4/K) oxalate as anticoagulant; (c) using undiluted blood with sequestrene as anticoagulant; (d), (b) and (c) repeated, but with an additional dilution with a 1 in 4 ratio of isotonic citrate/blood.

4. To investigate the effect on the result of delay between obtaining the blood sample and the carrying out of the E.S.R. estimation which is pertinent in a general practitioners' service where such a delay may amount to some seven to eight hours (9 a.m. to 4 p.m.).

5. To investigate less fully, and as a check on the excellent literature available, subjects such as: (a) the effect of contamination with spirit; (b) the preparation and concentration of the anticoagulant; (c) the cleanliness of the apparatus; (d) the relative accuracy of the apparatus used.

Materials and Method

The blood samples were obtained from male and female patients in two general medical wards of the Royal Infirmary, Edinburgh, and from a small number of patients attending the radiotherapy follow-up clinic

and the antenatal clinic in the Simpson Memorial Maternity Pavilion. The patients were selected for study because of the suspected abnormal level of their E.S.R. rather than for the nature of their illness, and the group was constituted as follows:

Age	Female (67 patients)	Male (68 patients)
10-19	3	6
20-29	1	7
30-39	9	4
40-49	15	5
50-59	14	12
60-69	13	12
70-79	11	11
80-89	1	0

Blood samples were obtained with minimal stasis and trauma from the antecubital vein, after cleansing of the skin with a small amount of methylated ether and allowing to dry. Dry sterilized siliconed 5-ml. and 20-ml. all-glass syringes fitted with syringe service $1\frac{1}{2}$ in. (3.8 cm.) 20-gauge hypodermic Luer needles were used throughout.

After removal of the needle from the 20-ml. taking syringe, the blood was gently added to a glass "Wintrobe" tube, previously cleaned with detergent and chromic acid and then rinsed clean and oven-dried. The tube, containing either sufficient solid sequestrene or NH_4/K oxalate to prevent 3 ml. of blood from clotting, was then stoppered with either a "supa-seal" cap or a red rubber bung. A further 3-ml. sample was also added to a tube without anticoagulant, and 2 ml. of this blood was then immediately withdrawn from it into a 5-ml. syringe and added to 0.5 ml. of isotonic 3.8% citrate solution in a further tube, thus constituting an approximation to the classical Westergren method. Immediately after filling, all the tubes were slowly inverted five times to ensure efficient mixing of the contents.

The blood samples were then examined at the laboratory within the next one to two hours, except when separate samples were set aside for various fixed times before examination, in order to determine the effect of storage deterioration on the E.S.R.

In the laboratory the samples underwent further mixing by inversion some 15 to 20 times, and portions were then taken from these tubes in one of three different ways:

1. Sufficient was sucked directly and undiluted into a Westergren pipette.

2a. 2 ml. was sucked into a 2-ml. bulb pipette and subsequently expelled into a second tube containing 0.5 ml. of isotonic citrate which had previously been accurately added from a microburette, the resulting 1 in 4 dilution being finally mixed by expelling from and sucking into the pipette at least twice.

2b. 2 ml. was sucked into a 2-ml. graduated pipette which had previously been used to introduce 0.5 ml. of isotonic citrate solution into an empty tube, and this blood was added to the citrate solution, mixing once more being achieved by sucking up and down as before, again producing a 1 in 4 dilution.

Once these diluted samples 2a and 2b had been prepared, Westergren pipettes were filled, each pipette requiring about

1 ml. of blood. One set of pipettes was filled in the standard manner and set up in the normal stand, using rubber cushions and spring-clips, while a second set of pipettes was suspended from hooks, using a very simple gimbal suspension (Fig. 2) designed by Dr. Duxbury, of Chase Farm Hospital, the distal ends of the tubes being blocked by cystoscope caps of soft red rubber.* Fig. 3 shows the Weyroc board for suspending the pipettes.

The blood was sucked into the pipette to above the zero mark and allowed to fall back in the usual way, thus satisfying any reflux effects, and, with the tube then held almost horizontally in the master-hand with the

FIG. 2.—Modified tube with simple gimbal mounting.

FIG. 3.—Weyroc board for suspending pipettes.

*These non-perforated caps were obtained from the Genito-Urinary Mfg. Co. Ltd., 33-34, Devonshire Street, London, W.1.

forefinger over the upper end, a red rubber cystoscope cap, previously moistened with water, was slipped over the lower end of the pipette until the lower end impacted on the cap with the air-escape hole uppermost to free the occluded air, and the pipette was then suspended on the hook provided.

All pipettes were then read at the end of one hour, accurately timed by a Smith stop-clock and a watch. The distance was then measured in millimetres from the bottom of the surface meniscus to the top of the red-cell columns, or to the upper surface of the buffy coat, or, as Westergren puts it, "to where the complete density appears to begin."

No adjustment for change of environmental temperature as recommended by Goldberg was made, but the effect of varying temperatures on the E.S.R. levels was noted throughout; indeed, no effect was found over the first eight hours of storage within the range 15–23° C. Beyond eight hours a rise in temperature, in particular, increased the error associated with storage.

Most of these experimental results were obtained with the pipettes suspended as described, and it was necessary to determine whether this practice was in itself liable to any major errors, of which the main one was thought to be the possibility of a lateral swinging movement of the suspended pipette as a result of draughts and other laboratory hazards.

This point was checked by running parallel experiments on one series of tubes hanging statically for the hour (static tubes), and on another series in which each tube was given a pendular movement over at least a

FIG. 4.—Wall-bracket for the Westergren pipette, used in a ward.

4-in. (10-cm.) arc by a sharp lateral blow, and the tube then allowed to swing until central once more (swinging tubes); this blow was repeated every 15 minutes during the hour, thus providing a degree of lateral movement that is more than could be reasonably expected under normal laboratory conditions. The results were virtually the same in the paralleled and duplicated sets of tubes.

It was thought that the blood diluent might just as well be normal saline as 3.8% sodium citrate solution, and this was then investigated, using parallel blood samples from two similar groups of patients. One line of duplicated samples was anticoagulated with sequestrene, then diluted with normal saline, while the other duplicated line was anticoagulated with sequestrene and then diluted with 3.8% citrate solution.

Results

The results of the various experiments are shown graphically. Figs. 5–11 show the comparison of methods on parallel blood samples with variables stated on each axis. The fixed line drawn in each case represents the 45 degrees line—that is, the line upon which the points should fall if the methods on the two axes gave identical results. Oxalated blood diluted with citrate was used as the reference standard throughout.

The results obtained when comparing normal saline with 3.8% sodium citrate as a diluent for the anti-coagulated blood are not shown graphically, but tabulated data at the end of the statistical section (Nos. 8–10) show that there is no practical difference.

FIG. 5.—Relationship between a close approximation of the "classical" Westergren method and the oxalate method adopted as a standard reference. The slight scatter was thought to be due to the differences between readings of the syringe and 2-ml. pipette markings.

FIG. 6.—Shows the increased scatter when undiluted oxalated blood is used.

Statistics

The statistical analysis of the results obtained is as follows: R = The correlation coefficient. (N.B. 1.0 would equal complete correlation.) R_u and R_l = The estimated upper and lower confidence limits to the correlation coefficient. (N.B. These represent the limits within which the true value might be expected to lie, using the number of estimations at our disposal.)

1. An approximation to the classical Westergren v. oxalated blood diluted with citrate (46 estimations).
 $R=0.994644$ $R_u=0.99728$ $R_l=0.98987$
2. Undiluted oxalated blood v. oxalated blood diluted with citrate (59 estimations).
 $R=0.844083$ $R_u=0.9069$ $R_l=0.7487$
3. Undiluted sequestrenized blood v. oxalated blood diluted with citrate (113 estimations).
 $R=0.8205167$ $R_u=0.8741$ $R_l=0.7487$

FIG. 7.—Shows a situation similar to Fig. 6 when undiluted sequestrenized blood is used.

FIG. 8.—Shows the good correlation obtained between the adopted method using sequestrenized blood diluted with citrate and the standard reference method, and therefore, both with the adaptation of the classical method of Fig. 5 and, on the evidence of Goldberg's paper, with the true classical method.

4. Sequestrenized blood diluted with citrate v. oxalated blood diluted with citrate (132 estimations).
 $R=0.9988845$ $R_u=0.999$ $R_l=0.99818$
5. Sequestrenized blood diluted with citrate v. sequestrenized blood diluted with citrate (105 estimations).
 $R=0.9992154$ $R_u=0.99945$ $R_l=0.99878$
6. Undiluted sequestrenized blood v. undiluted sequestrenized blood (92 estimations).
 $R=0.896117$ $R_u=0.9302$ $R_l=0.8456$
7. Suspended and static sequestrenized blood diluted with citrate v. sequestrenized blood diluted with citrate suspended and swinging (108 estimations).
 $R=0.999133$ $R_u=0.99945$ $R_l=0.99878$
8. Sequestrene blood with saline v. sequestrene blood with saline (87 estimations).
 $R=0.9986601$ $R_u=0.99918$ $R_l=0.99777$
9. Sequestrene blood with citrate v. sequestrene blood with citrate (87 estimations).
 $R=0.9991699$ $R_u=0.99945$ $R_l=0.99878$
10. Sequestrene blood with 3.8% sodium citrate v. sequestrene blood with normal saline (174 estimations).
 $R=0.994476$ $R_u=0.99595$ $R_l=0.99248$

FIG. 9.—Shows the reproducibility of the Fig. 8 method.

FIG. 10.—Shows the unreliability of using undiluted blood samples; for, with both axes varying, the wide scatter obtained cannot be correlated. Nevertheless, the degree of scatter is impressive.

Conclusions

It was concluded that the best method of determining the E.S.R. is to dilute 2 ml. of a sequestrenized blood sample with one-quarter its volume of isotonic 3.8% sodium citrate and then to examine the "fall" in a freely suspended tube. This conclusion was based on a comparison of E.S.R. values obtained using various methods against a common standard method in which oxalated blood diluted with one-quarter its volume of 3.8% sodium citrate solution was used. The adopted

FIG. 11.—Shows the absence of effect of a lateral swinging movement on the tube as described in the text. This was an interesting and perhaps surprising result.

FIG. 12.—One of a series of graphs showing that over an 8 to 12-hour storage period the changes observed in the E.S.R. value are clinically insignificant, but that, on the whole, the rate of fall tends to lessen as the sample becomes older. (N.B.—Each line represents one blood sample followed under the stated conditions.)

sequestrenized method and the standard method gave virtually identical results, and consequently, if the paper by Goldberg and Conway (1952) comparing the classical Westergren method with this so-called modified classical Westergren method is accepted as reliable, the results can be satisfactorily correlated with Westergren's classical standards, the ones in everyday use.

It was also concluded that:

1. The method of using whole anticoagulated blood in a Westergren pipette is inaccurate, and, whilst admitting that the extra step of dilution is a time-consuming addition, it is one that is fully justified, as the values obtained when using undiluted blood are not reliable or repeatable, and E.S.R. estimations on parallel blood samples may often differ by as much as 20 mm. from patients with mild clinical symptoms. In particular patients, especially those showing major changes in blood-protein values, the differences may be much greater; one blood sample observed in this series produced parallel values of 6 and 49, and still greater differences have been noted in patients outside this particular set of experiments.

2. Upon consideration of the preceding paragraph, it is obvious that the use of undiluted anticoagulated blood makes an E.S.R. result of little value as a diagnostic screening aid in an out-patient clinic or when an inter-laboratory comparison of results is being made.

3. The methods using anticoagulated undiluted blood have never been standardized, and it is unjustifiable to assume that they have the same normal values as the "classical" method.

4. It has been shown on physico-chemical grounds and the evidence published that it is incorrect to use undiluted blood to study a phenomenon of this kind in a tube whose bore is less than 4 mm. (Westergren and Wintrobe pipettes in general use have a bore about 2.2 mm.–2.6 mm.).

We chose sequestrene as the standard anticoagulant for the following reasons:

1. It was already in use in our laboratory as a dry and adhering solid prepared in glass Wintrobe tubes for storing 3-ml. blood samples for routine investigations. (N.B.—This volume, 3 ml., is to be increased to 4 ml. in our laboratory to enable repeat tests to be carried out on samples producing anomalous results, or to allow for mishaps occurring during the laboratory investigations.)

2. It enables platelets to be counted on venepuncture blood, and red cells to be counted by an electronic counter—a situation quite impossible with the older oxalate mixture, as the counter cannot discriminate between insoluble calcium oxalate crystals and cells, the resulting error of this being in the order of millions per cubic millimetre.

3. The overall deterioration of such blood samples is less than with other anticoagulants, and especially is this so with reference to cell nuclei and membranes of markedly abnormal "bloods" when morphology is often of prime importance. This aspect of reduced deterioration of blood samples with time is also a very relevant factor if the hospital service is providing external facilities for general practitioners, who, by the nature of their work, often cannot get their blood samples into the laboratory as quickly as can a hospital ward.

4. Within the temperature range 15–23° C., a specimen of sequestrenized blood yields an accurate E.S.R. estimation for at least eight hours after venepuncture, though, ideally of course, the shorter the time that elapses the better. It is felt, however, that thereafter a stored sample tends to give a falsely low result, but this is not markedly so until after 24 hours have elapsed.

5. There is a high degree of repeatability of results.

6. Two minor points of more scientific than clinical import in the use of sequestrene are (a) a shortened phase 1,

and (b) a reduced tendency for cells to shrink, thereby increasing the accuracy of both the E.S.R. and the packed cell volume.

Sequestrene acts as an anticoagulant by chelating the calcium ions of the blood, through the action of its carboxyl (COOH) groups to form a soluble non-ionized salt. It is used in a concentration of 1–1.5 mg./ml. of blood, and it is suggested that if the higher figure is employed the chance of clotting occurring in the presence of inadequate mixing of blood and sequestrene is minimized, and it also permits a wider degree of error in measuring the 3 ml. of blood—a practical point in a busy ward.

Sequestrene is available as the di-potassium or the di-sodium salt, the solubilities in water being 20% W/V and 6% W/V respectively. We therefore chose to use the di-potassium salt as a 5% aqueous solution. The crystalline salt for preparing this solution can be obtained from the General and Pharmaceutical Co. Ltd., Judex Works, Sudbury, Middlesex.

Unfortunately, to prepare the 5% solution takes some two hours, owing to its slow solubility, and, likewise, the time taken to evaporate the solution to dryness in the Wintrobe tube at room temperature takes about five days, but this last procedure must be quite complete or a curious hygroscopic phenomenon occurs and the crystals liquefy once more. However, drying can be accelerated by the use of judicious heating (45° C. in an x-ray drying cupboard for two days) without any apparent deleterious effect on the anticoagulant properties of the chemical, a result which both the manufacturers and our chemistry department colleagues confirmed.

If excessive heat is used to dry the sequestrene a greater amount of the water of crystallization will be driven off, and this causes a tendency to form a fused "button" in the bottom of the tube, resulting in a reduced surface area of contact, and thereby necessitating greater care in the initial mixing of the anticoagulant and blood.

The Westergren pipette was chosen because: (a) it is in wide general use, (b) it requires only about 1 ml. of blood, and (c) its size makes it easy and practicable to use. The only other serious alternative, the Wintrobe pipette (100 mm. long), has, because of its small bore, been fully discredited in the literature, and, in spite of the convenience of being able to follow an E.S.R. estimation with a P.C.V. in the same tube, it should not be used.

When a Westergren pipette was made to deviate from the vertical in our experiments, a very real experimental error was introduced. This finding was fully supported by (a) similar findings in the available literature, and (b) the marked difference in the standard values as found by the rapid method of E.S.R. determination using a fixed angle tube as compared with a static vertical tube. These points suggested very strongly to us that the adoption of a freely suspended, and thus vertical, pipette would be a considerable advance.

This was achieved as described above, and the only novel variant thereby introduced that could not affect the static tube customarily used was the possibility of lateral movement caused by draughts and the like. In fact, quite strong draughts do not move the pipettes at all, but it was felt necessary to see if lateral movements would affect the value of an E.S.R. reading.

The adoption of the suspended Westergren pipette provides a method which is readily adaptable to most situations, from a large central laboratory to a small back room, and is cheaper, simpler to use, and easier to read than the conventional method.

Other facts noted during this series of experiments were:

1. Relatively dirty pipettes—that is, those merely washed in tap-water and allowed to dry—did not seem to affect the

accuracy of the readings to any great degree, an observation of importance for a busy ward, where chemical cleaning of tubes is not always practicable.

2. Varying the blood/anticoagulant dilutions had a relatively marked effect on the E.S.R. and hence the 1 in 4 dilution should be carefully made, using, if possible, microburettes and accurate pipettes.

3. Contamination with spirit, etc., at any stage in the process from venepuncture to estimation must be prevented.

4. A few bubbles in the Westergren pipette seem to have very little effect on the E.S.R. itself, provided they are present at the top of the blood column or remain static at the bottom. Should they be present at the top of the blood column, their volume must be deducted in reading the E.S.R., as they, in themselves, do not form a part of the fluid column.

5. The estimation of the E.S.R., using general hospital syringe service syringes and done in a central haematological laboratory, appears to benefit in that it becomes more repeatable and at the same time relieves the houseman of humdrum work he need not be required to perform.

6. Westergren's "classical" method is not as accurate as the modified classical method of Goldberg, because in the latter: (a) the error introduced by immovable bubbles in the syringe is avoided, and (b) the 2-ml. pipette used as described for diluting blood is more accurately read than the plunger mark of a glass syringe.

7. The ability to pick up the suspended Westergren pipette from its hook greatly reduces the breakage rate of pipettes, and facilitates the reading of the result.

8. The general practice of cleaning glass pipettes using the sequence water–alcohol–ether–air, in contrast to water–acetone–air, was found to be unnecessary for the following reasons: (a) it is much more expensive, (b) it requires an extra container for the fluids, and (c) the amount of inflammable vapour which reaches the room and the drain is greater with alcohol–ether than with acetone. (N.B.—Neither sequence affects the E.S.R., and acetone is fully miscible with water.)

Considerations

From a review of the literature and supported by these experimental results, a series of concepts was deduced for guidance in interpreting an E.S.R. value.

1. That Westergren's original normal E.S.R. ranges (normal male adult, 0–5 mm. per hour; normal female adult, 0–10 mm. per hour) were felt to be too narrow, and that a further 5 mm. should be added to each. Within these ranges low readings—that is, 1 mm. and 2 mm.—are abnormal.

2. That in adults over 50 years, higher values may be normal.

3. That in pregnancy, which normally causes a marked rise in E.S.R., the normal range should be up to 45 mm. in one hour.

4. That normal neonates have a low E.S.R.—about 2 mm.—by the standard method.

N.B.—The micromethod of determining the E.S.R. of an infant, using a capillary tube and blood obtained by heel stab, is relatively inaccurate, and where an abnormal value is obtained (based on locally accepted norms) the estimation should be repeated by the suggested standard method.

The normal E.S.R. range in use at any particular centre will be modified by local conditions. The adult West African, in a centre using heparin as anticoagulant for the blood samples, is credited with having a normal range of 5 to 30 mm. per hour. This may possibly be due to masked endemic disease, in addition to the effects of the heparin.

Summary

The various methods of estimating the E.S.R. employed in the Edinburgh group of hospitals have been investigated and found wanting. A new method has been suggested, employing sequestrene as the anticoagulant and using a suspended Westergren pipette in place of the static pipette usually employed. This new method has been checked and found satisfactory, and has been in use over the past 18 months in the Royal Infirmary, Edinburgh.

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ORTHO-CRESOTINATE AND DIABETES MELLITUS

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Aspirin in full therapeutic doses relieves mild diabetic symptoms, lowers the fasting blood-sugar level, and reduces glycosuria and ketonuria in patients with mild to moderately severe diabetes mellitus (Reid, Macdougall, and Andrews, 1957). One of the disadvantages of aspirin therapy, however, is the fairly frequent occurrence of undesirable side-effects, such as tinnitus and deafness, with the high dosage of the drug required to achieve satisfactory control of the diabetes.

Ortho-cresotinic acid.

Stockman (1912) observed that sodium ortho-cresotinate (see Formula) was as effective as sodium salicylate in treatment of rheumatic fever, but even with

high dosage it did not cause deafness or tinnitus. The effect of ortho-cresotinate in diabetes mellitus has been investigated to find out whether it has a similar hypoglycaemic action to that of salicylate without its undesirable side-effects.

Nine patients with diabetes mellitus were treated with ortho-cresotinate, and clinical particulars are shown in Tables I and II.

TABLE I.—Clinical Particulars of Group 1. Patients Previously Treated by Dietary Restriction Alone

Case No.	Age	Sex	Weight (kg.)	Duration of Diabetes	In-patient Diet	
					g. Carb./Day	Total Cals./Day
1	68	F	57.7	3 years	150	1,600
2	59	F	72.9	1 month	120	1,300
3	60	F	71.5	8 years	150	1,500
4	57	F	56.5	2 "	150	1,700
5	68	F	52.9	3 months	150	1,700
6	55	M	59.5	3 "	148	1,600

TABLE II.—Clinical Particulars of Group 2. Patients Previously Treated by Diet and Insulin

Case No.	Age	Sex	Weight (kg.)	Duration of Diabetes	Diet		Insulin Requirement (Units/Day at End of Control Period)
					g. Carb./Day	Total Cals./Day	
7	49	F	56.5	1 year	195	2,100	28 I.Z.S.
8	49	F	50.7	10 years	195	2,100	50 "
9	37	M	79.9	6 "	200	2,000	54 "

The first group of six patients (group 1) had been treated previously by dietary restriction only for periods varying from one month to eight years, but control of the diabetes had not been satisfactory, as indicated by the occurrence of mild diabetic symptoms in five of the patients, and the presence of persistent glycosuria and elevated blood-sugar levels in every patient. Two patients (Cases 2 and 3) were overweight at the beginning of the investigation; the other four were at or below their optimum weight. The second group of three patients (group 2) had already been treated by diet and insulin before the investigation. Their insulin requirement when admitted to hospital ranged from 28 to 88 units of insulin zinc suspension (I.Z.S.) daily.

Methods of Investigation

All patients were admitted and detained in hospital for the duration of the investigation. Treatment was controlled and the effect of the drug determined by frequent estimations of the fasting blood-sugar and serum ortho-cresotinate levels and daily measurement of sugar and ketone output in 24-hour urine. Every patient in group 1 was first treated by dietary restriction until the fasting blood-sugar was stable. This control period varied from 7 to 13 days. The daily carbohydrate intake, ranging from 120 to 150 g. was kept constant throughout both the control and treatment periods. The diet contained 65 to 79 g. of protein, and 65 to 85 g. of fat, and the total calorie content ranged from 1,300 to 1,700 a day. At the end of the control period of dietary restriction the patients were given a course of ortho-cresotinate which lasted for at least two to three weeks.

The three patients in group 2 were treated initially by dietary restriction and insulin for a control period of 10 to 24 days. During this time the daily dosage of insulin was altered if necessary to stabilize the fasting blood-sugar. After the control period a course of ortho-cresotinate, lasting for two to three weeks, was started and insulin administrations continued. The dose of