Because of the wide range of plasma factor VIII concentrations found in normal donors, and the inaccuracy of assays on concentrated material, the best basis on which to calculate the appropriate dose of concentrate for a given situation is the factor VIII concentration of the original plasma from which the cryoprecipitate was prepared. If this is known the dose can be calculated without waiting for the result of an assay performed on the reconstituted material, applying a correction factor to account for the expected recovery, as described above. In practice, however, it may not always be possible to assay the original plasma, and in such a case the following formula may be used as a rough guide:

$$\text{No. of packs required} = \frac{\text{Factor VIII rise intended (\%)} \times \text{body wt. (kg.)} \times 4}{1{,}000}$$

This is based on the assumptions that a pack of fresh plasma contains 250 units of antihaemophilic globulin, and that the overall in-vivo recovery is not less than 50%, so that 1 unit of antihaemophilic globulin per kg. raises the patient's factor VIII concentration by not less than 1% (see Clinical Results). When the concentration to be achieved is critical, as in major bleeding episodes or to cover operative procedures, it is advisable to check the potency of the material by assaying the concentrate itself. We have found the use of supernatant plasma as the initial diluent for the cryoprecipitate to give more reproducible results in our assay system than other modifications which we have tested.

Summary

Antihaemophilic globulin concentrate was prepared from 278 packs of fresh plasma by Pool's cryoprecipitation method, and from a further 103 packs by a modification of this method, involving more rapid thawing of the plasma. These concentrates were used for the treatment or prevention of bleeding on 59 occasions in 23 haemophilic patients, including two major surgical operations.

The mean overall recovery of factor VIII in the patient's circulation, relative to the original plasma from which the concentrate was prepared, was 43% for concentrates prepared by Pool's original method and 61% in the case of those prepared by the rapid modification. This improvement in recovery appeared to be due to a reduction in loss of factor VIII during the faster thawing procedure.

In concentrates prepared by the rapid method the factor VIII was concentrated over twentyfold, and the fibrinogen over tenfold, relative to the total protein. Concentrates were stable on storage at −30° C. for at least 10 weeks. The half-life of factor VIII in the circulation of haemophilic recipients of cryoprecipitate antihaemophilic globulin varied from 6 to 14 hours.

Excellent clinical results were achieved in all patients treated, except two who had circulating factor VIII inhibitors; one of these developed during treatment with cryoprecipitate antihaemophilic globulin. No other side-effects or reactions were observed. The advantages of this type of human antihaemophilic globulin preparation are discussed, and a guide to the calculation of dosage is given.

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Normal Erythrocyte Sedimentation Rate and Age

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It has become more and more important to establish reliable normal values for the biological variables used in clinical medicine. The widespread use of, and demand for, health surveys of population groups has made it necessary to have well-defined limits for "normality" applicable to mass examinations. The increasing number of old people in the hands of the medical profession all over the world also make it necessary to have *age-grouped* normal values. Values now commonly in use have been employed to distinguish between health and well-established disease in individual patients and are generally not correlated to age.

At least in the Scandinavian countries, the erythrocyte sedimentation rate (E.S.R.) performed by the Westergren method has been one of the most commonly employed tests to separate "disease" from "health." Westergren's original normal values (men 3 mm., women 7 mm./hr.) are now generally thought to be too low to be of practical value, especially in older people. Many have had the impression that there is a general increase of the E.S.R. with age, but it is always difficult, sometimes impossible, to state whether such an increase is due to hidden disease or to "normal" age factors. A large group of healthy people—in active work—regularly examined at a health survey centre offered an excellent opportunity to study the influence of age on the E.S.R.

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Material and Methods

The material was obtained from a health survey centre (Företagens Hälsokontroll, Stockholm). This centre makes regular examinations, at yearly intervals, of employees of various companies that have a contract with the centre. Those who come for their routine visits are all engaged in active work

and are subjectively healthy. Most of those included in this investigation have been followed for several years, both before and after this particular study was started.

The subjects were examined physically and routine tests on blood and urine were made.

To avoid, so far as possible, latent atherosclerotic disease, those whose parents suffered or died from ischaemic heart disease or cerebral vascular accidents have been excluded. Apart from a normal physical examination and exclusion of those pregnant and those with a previous history of diabetes, ischaemic heart disease, or thyroid or biliary disease, the following criteria were used for the selection of normals.

Haemoglobin	...	Men 12.8–16.8 g./100 ml. Women 11.2–16.8 g./100 ml.
Blood pressure	...	Systolic <170 mm. Hg Diastolic <100 mm. Hg

(Blood-pressure readings obtained on the first visit without rest)

Urine ... ... No sugar or protein present

All patients attending the health survey centre during one year formed the original material, approximately 6,500 persons. Selection according to the criteria mentioned was done by data-processing. There remained 1,457 men and 1,021 women, total 2,478 persons, who fulfilled these criteria. Persons belonging to the extreme age groups (below 20 or over 70) were further excluded because of the small numbers in these groups. Eight men and nine women were excluded for this reason—11 from the young age group and six from the old. Forty-five persons had an E.S.R. greater than 25 mm./hr. The charts for all these have been scrutinized. Three further exclusions were made—namely:

Man aged 52 with an E.S.R. of 60 mm./hr.—remaining high throughout several years (observation time six years).

Woman aged 67 with an E.S.R. of 40 mm./hr.—though fluctuating, always elevated (observation time nine years).

Man aged 64, recent diagnosis of pernicious anaemia—remission not complete at time of examination.

Twenty-two subjects (four men and 18 women) apparently had a recent respiratory infection at the time of the examination, as ascertained from their own statements and the finding of a normalized E.S.R. on control examination one month later. The original high E.S.R. values for these persons have, however, not been excluded ; nor have those of the 20 persons (four men and 16 women) who had a persistently slightly raised E.S.R. (above 25 mm./hr.), and who were followed up in most instances for several years without any explanation being found. The final material thus consists of 1,447 men and 1,011 women, a total of 2,458 persons.

A further group was also selected to make it possible to study the correlation between E.S.R. and haemoglobin values (see Fig. 2). For this purpose no subject was excluded because of a low haemoglobin value. This larger group consists of 2,547 persons (1,508 men and 1,039 women).

Results

The variation of the E.S.R. with age is shown in Fig. 1. It will be seen that the E.S.R. increases with age, in both men and women. This increase is highly significant statistically in both sexes ($P<0.001$). The increase in men is constant through the age groups, averaging 0.85 mm./hr. per five-year group. The values in women are higher than in men in all age groups ; furthermore, while the increase up to the age of 50 is fairly constant and of the same degree as in men (0.53 mm./hr. per five-year group), the increase after the menopause is much greater (2.8 mm./hr. per five-year group).

The correlation between age and haemoglobin values has also been tested. First this was done in the larger group, where, as stated, no exclusions had been made because of low haemoglobin values. The results are given in Fig. 2. The haemoglobin values in men show a slight decrease. The difference between the mean values in the youngest and the oldest age group is 0.7 g./100 ml., this difference, however, being highly significant ($P<0.001$). In women, on the other hand, the haemoglobin values are constant, or actually show a slight increase with age ($0.05<P<0.01$). When persons with low haemoglobin values are excluded (for limit values see above) the results obtained are the same in men—a small though significant decrease with age—but in women no change is found.

The larger group, without exclusions, was studied mainly to see whether any correlation could be found between the E.S.R. and the haemoglobin values. The results and the statistical calculations show that such a correlation exists and that it is highly significant in both sexes. The calculation of partial correlation coefficients shows that this correlation exists regardless of the observed changes in haemoglobin values with age.

The correlation between the E.S.R. and the haemoglobin values is still to be found even when persons with low haemoglobin values have been excluded. Thus even in the narrow range of normal haemoglobin values such a correlation exists.

Discussion

Different opinions have been held whether the E.S.R. varies with age or not, but actual studies are sparse. Westergren, who was the first to lay down normal values, stated that, strictly speaking, the normal limit at one hour should be taken as 3 mm.

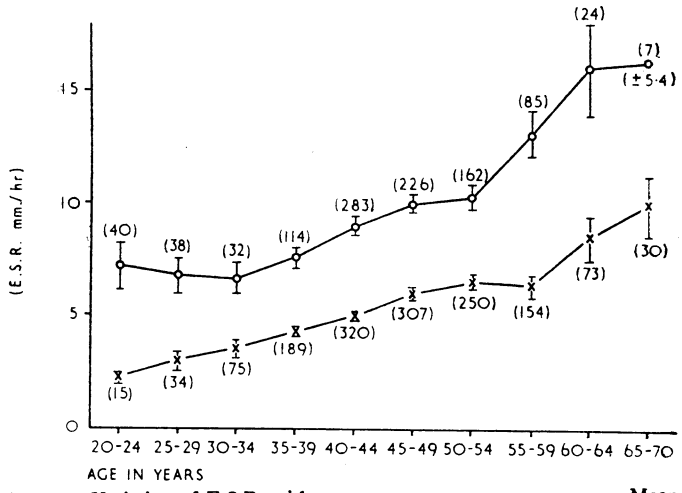

FIG. 1.—Variation of E.S.R. with age. o—o = women ; x—x = men. Mean values ± standard error (mm./hr.). The number of cases in each age group is given in parentheses.

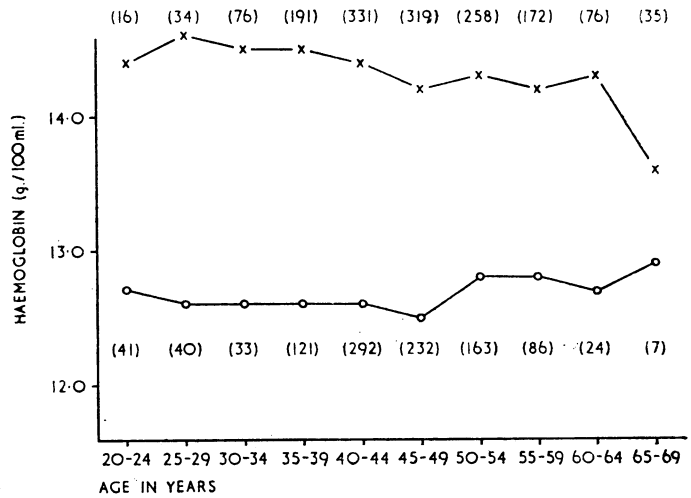

FIG. 2.—Variation of haemoglobin with age. o—o = women ; x—x = men. Mean values (g./100 ml.). The number of cases in each age group is given in parentheses.

in men and 7 mm. in women. Later he stated that an upper limit of 5 mm. for men and 10–15 mm. for women was often better suited to the requirements of clinical practice (Westergren, 1957). He also added that the higher values were especially applicable to patients over 50. Eckerström (1949) examined 100 men and 100 women aged over 70 and found that he could not verify the theory that a high E.S.R. would accompany old age. Wilhelm and Tillisch (1951) examined 565 consecutive records of patients in good health and found that the mean E.S.R. increased with age. Borchgrevink *et al.* (1965), in a series of blood donors, found that 18% of all people in age group 51–60 had an E.S.R. above 15–20 mm./hr. Dahlberg and Josephson (1955) found the mean E.S.R. for men and women under 50 to be 4 and 7 mm./hr. respectively and for men and women between 70 and 80 to be 12 and 20 mm.

The results obtained in this study show that there is a definite though small increase with age. The normal range calculated from the results is shown in the Table.

Suggested Normal Ranges for E.S.R. in Various Age Groups (mm./hr.)

	Age Group	$\bar{x}$	S.D.	Range	
				$\bar{x} \pm 2$ S.D.	$\bar{x} \pm 2{\cdot}5$ S.D.
Men	20–49 ..	5·0	4·0	0–13	0–15
	50–69 ..	7·0	5·8	0–19	0–22
Women	20–49 ..	8·8	6·1	0–21	0–24
	50–69 ..	11·8	8·0	0–28	0–32

A close correlation exists between haemoglobin values and E.S.R., even when the haemoglobin values are within the normal range. A decrease of the haemoglobin value, though only within the chosen normal range (16.8–12.8 g./100 ml.) in a man would correspond to an increase in the E.S.R. approximately from 3 to 8 mm./hr. This would imply that part of the difference between men and women as regards E.S.R. values might be explained by differences in haemoglobin values. The mean difference between men and women in the present series is 1.7 g./100 ml., which corresponds to an E.S.R. difference of approximately 2 mm. Differences in haemoglobin values cannot, however, explain the whole difference between E.S.R. values in men and women, nor the increased difference between men and women after the age of 50. During that age period the difference in haemoglobin values in the present series tends to diminish instead of increase.

In spite of some renewed interest in the mechanisms of the E.S.R. it is still not known what factors are responsible for the aggregation and sedimentation of erythrocytes. The importance of fibrinogen had already been shown by Fåhraeus (1921) and Westergren (1957), and generally has not been disputed. Ruhenstroth-Bauer (1961), however, stated that an increase in fibrinogen and gammaglobulin is only coincidental to a high sedimentation rate and not its cause. It has also been stated that carbohydrate-rich serum proteins other than fibrinogen, especially the alphaglobulins, might influence the E.S.R. (Stary *et al.*, 1951). Experiments have shown that splitting off the sialic acid from carbohydrate complexes of such proteins with neuraminidase might alter the sedimentation rate (Stickl and Böcker, 1959).

How do the serum proteins behave with increasing age? In respect of fibrinogen only a few studies have been made. Schulz (1951) found higher contents in women than in men, and in both sexes an increase with age, slow before the age of 40, more rapid in higher age groups. Steinmann (1964) examined more than 400 subjects, and found an increase in fibrinogen with age. It should be noted, however, that the young subjects were healthy volunteers, whereas the older people were mainly patients. With regard to total protein and electrophoretically separated protein fractions, it has repeatedly been found that old people have essentially the same total protein and protein pattern as young ones (Böttiger and Carlson, 1960; Böttiger and Holmström, 1964; Lyon *et al.*, 1964). Ultracentrifugal studies of serum from normal men did not reveal any changes with age, nor did studies of protein-bound carbohydrates in serum (Böttiger and Carlson, 1966). It is thus difficult to point to any special factors in the protein pattern that would explain the increase of the E.S.R. with age.

The more rapid increase in E.S.R. values for women after the age of 50 would point to hormonal influences. Such influences could act indirectly by altering the amount of specific plasma proteins. Studnitz and Nyman (1957), among others, have demonstrated that treatment of women with androgens increases the serum level of alpha$_2$-globulins. Nyman (1959) further found a small increase of serum haptoglobin values in aged people. Houssay and Blumenkrantz (1964) studied the effect of sex hormones on the serum glycoprotein content in the rat. They found that oestradiol increased the content of serum glycoproteins in castrated female rats and testosterone decreased the values in castrated male animals. In this connexion the increase in overt ischaemic heart disease in postmenopausal women should also be mentioned.

A possible hormonal influence on the E.S.R. has also been shown by Larsson (1962), who found a negative correlation between the E.S.R. and protein-bound iodine values in hypothyroidism. He also found an increase of the beta$_2$-globulin fraction, which contains the β-lipoprotein known to be increased in hypothyroidism. He concludes that the E.S.R. is dependent not only on proteins but also perhaps on lipids.

Summary

The E.S.R. (Westergren method) has been studied in 2,500 healthy men and women aged 20–70. In both sexes the E.S.R. significantly increases with age. In all age groups the values were higher in women than in men.

The increase in E.S.R. was constant and of the same degree in men through all age groups and in women up to the age of 50. In older women the E.S.R. increased more rapidly. The altered rate of increase at the menopause would suggest a hormonal influence on the E.S.R.

There is a close negative correlation between the E.S.R. and the haemoglobin content; this can be demonstrated even when only persons with haemoglobin values within the normal range are studied.

The results would indicate that the upper normal limits for E.S.R. should be given as follows (mm./hr.). Below the age of 50: men 15, women 25. Above the age of 50: men 20, women 30.

Företagens Hälsokontroll allowed us to use their material, and the Folksam Insurance Group put data-processing time at our disposal. Our thanks are due to these institutions for their valuable help, without which this study could not have been made.

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