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"The future depends largely on our ability successfully to develop and market new products in response to a steadily changing incidence of disease. Some of these changes are due to factors beyond our control; others are influenced by our own activities. . . . These considerations help to explain the vital part played by technical, as distinct from economic, competition in our industry. However efficient we may be in producing and selling our existing products, the discovery of an effective new medicament or, for example, a more economical synthesis of an existing medicament, can render obsolete a product or group of products that may have taken us years to perfect and may have involved the investment of large sums in providing specialized plant and the skilled personnel necessary to its operation. Against such a background of technical competition we must view with considerable concern the relatively small expenditure on medical and pharmaceutical research in the United Kingdom compared with the weight of effort now being put into such research overseas, particularly in the United States, the Common Market countries, and Japan." (Sir Harry Jephcott, 25th Annual General Meeting, Glaxo Laboratories, Ltd.)

SEDIMENTATION RATE OF STORED BLOOD, USING SEQUESTRENE AS THE ANTICOAGULANT

BY

IAN D. MELVILLE, M.B., F.R.F.P.S., M.R.C.P.

Medical Registrar, Royal Infirmary, Glasgow

AND

BASIL M. RIFKIND, M.B., Ch.B.

Senior House Officer, Royal Infirmary, Glasgow

It has been shown that a modification of Westergren's method for the estimation of the erythrocyte sedimentation rate (E.S.R.), using blood treated with sequestrene, gives results similar to those of the customary method, whether the E.S.R. is estimated immediately after the withdrawal of the blood or when the blood has been stored in the refrigerator for 24 hours (Melville and Rifkind, 1959).

Further work has been carried out to find the effect on the E.S.R. of longer storage of blood samples in the cold, and of storage of samples at room temperature for various periods.

Material and Methods

Blood samples were taken from patients in medical wards of a general hospital. Some samples were taken deliberately from patients known to have raised E.S.R.s. The sequestrene used as anticoagulant was the disodium salt of ethylenediamine tetra-acetic acid, at a concentration of 1 mg. per ml. of blood. Blood so treated is here termed "sequestrene blood."

For the estimation of the E.S.R. the modified Westergren method (Melville and Rifkind, 1959) was used. Mixing of the sequestrene blood and of the citrate-blood suspensions was carried out for at least two minutes. Whenever possible the E.S.R. estimations were made in duplicate, but in some cases when a prolonged study was to be made, only one estimation was done so that blood could be conserved. The readings were taken to the nearest millimetre after one hour. The room temperature of the laboratory was measured at intervals, and was usually in the range of 18 to 20° C. Blood from the refrigerator was always allowed to warm up to room temperature before the E.S.R. estimations were made.

Experiments

The E.S.R. of sequestrene blood measured immediately after withdrawal of the blood will be termed the "immediate E.S.R." Experiments were designed to compare the immediate E.S.R. of a number of samples with their E.S.R.s after they had been stored for various times, as follows: experiment 1, storage for 48 hours at 4° C.; experiment 2, storage for 24 hours at room temperature; experiment 3, storage for six hours at room temperature. In these three experiments the blood was not disturbed during the storage.

Experiment 4 was designed to find if there was a significant change of the E.S.R. of a sample during the

six-hour period after venepuncture. In this study the blood from the patient was divided into a number of smaller samples which stood at room temperature. One of these was taken for E.S.R. estimation each hour, if possible, until the blood was used.

The effect on the E.S.R. of intermittent mixing of the blood sample was studied in experiment 5: 20 to 30 ml. of sequestrene blood was taken into a universal container, and hourly estimations of the E.S.R. were made whenever possible, until all the blood had been used. The sample was mixed thoroughly before each E.S.R. estimation.

Results

Table I shows the results of experiment 1. In 13 of the 18 tests there was considerable retardation of the E.S.R. after the blood had been stored in the cold for 48 hours.

Table II shows the results of experiment 2. In 14 of the 15 tests retardation of the E.S.R. was marked after storage of the blood at room temperature for 24 hours.

The comparison of the immediate E.S.R. with the E.S.R. of blood left for six hours at room temperature is shown in Table III. No significant retardation occurred with any of the tests.

TABLE I.—Comparison of the Immediate E.S.R. with the E.S.R. of the Sample Kept at 4° C. for 48 Hours

Sample No.	Immediate E.S.R.* (mm.)	48-hour E.S.R.* (mm.)
1	3	2.5
2	1	1
3	25.5	16
4	1	1
5	15	9.5
6	27	22.5
7	25.5	14.5
8	29	22
9	48	39
10	24	10
11	32	19
12	41.5	8.5
13	70	72.5
14	27	27.5
15	80	67
16	25	17
17	6	4.5
18	53	40.5

* Each figure is the mean of two estimations.

TABLE II.—Comparison of the Immediate E.S.R. with the E.S.R. of the Sample Kept at Room Temperature for 24 Hours

Sample No.	Immediate E.S.R.* (mm.)	24-hour E.S.R.* (mm.)
1	24.5	7.5
2	30.5	16
3	36.5	17
4	28	29.5
5	5	2
6	28	9.5
7	22.5	9.5
8	62.5	22
9	15.5	8
10	29.5	18.5
11	7	4
12	14	10
13	5	3
14	71	13
15	13.5	7.5

* Each figure is the mean of two estimations.

TABLE III.—Comparison of the Immediate E.S.R. with the E.S.R. of the Sample Kept at Room Temperature for 6 Hours

Sample No.	Immediate E.S.R.* (mm.)	6-hour E.S.R.* (mm.)
1	13	11
2	73	69
3	30	26
4	19	18.5
6	12.5	15
6	71	73
7	*3	4
8	30	29.5

* Each figure is the mean of two estimations.

TABLE IV.—E.S.R. in the 6-Hour Period Following Withdrawal of Blood. Small Quantities of the Initial Sample Allowed to Stand Undisturbed Until Used for the Estimations

Sample No.	Immediate E.S.R.* (mm.)	*E.S.R. (mm.) at Hourly Intervals After Withdrawal of Blood					
		1	2	3	4	5	6
1	7.5	7	7	7	7	6	—
2	71	73	—	70	74	72	73
3	3	—	3	4	3	—	5
4	30	—	32	31	32	30	30

* Each figure is the mean of two estimations.

TABLE V.—E.S.R. at Intervals Following Taking of Blood, When the Entire Sample is Mixed Thoroughly Before Each Estimation is Made

Sample No.	Immediate E.S.R. (mm.)	E.S.R. (mm.) at Hourly Intervals After Taking Blood										
		1	2	3	4	5	6	7	8	9	10	11
1	21	—	20	20	19	21	22	22	17	18	17	19
2	70	69	—	70	70	78	77	71	—	—	—	—
3	35	—	—	31	—	25	36	37	32	30	31	—
4	8	—	10	—	10	9.5	—	10	—	—	—	—
5	42	—	—	—	53	—	—	47	47	—	—	—
6	50	51	49	50	46	48	—	—	—	—	—	—
7	77	79	78	77	76	—	—	—	—	—	—	—
8	2	2	2	2	2	2	—	—	—	—	—	—

Table IV shows the results of experiment 4. No significant retardation of the E.S.R. occurred by the time each test series ended, and throughout the series, at the intervening times, no undue change in the sedimentation rates took place.

The results of experiment 5 are shown in Table V. It was not always possible to carry out hourly estimations, and this accounts for the blanks in some of the series. The E.S.R. after five or six hours' standing is not significantly different from the immediate E.S.R.

Discussion

The aim of this work was to find how long sequestrene blood could be stored, and under what conditions, before marked retardation of the E.S.R. occurred. The basic assumption is made that the correct E.S.R. of a blood sample is that found immediately after the blood is withdrawn. The physician is interested in learning the highest level of the E.S.R., and knows that retardation of the E.S.R. which follows storage or standing of the blood will be misleading.

Our previous work demonstrated that 24-hour refrigerated storage of sequestrene blood did not appreciably affect the E.S.R. The present investigation shows that refrigerated storage for 48 hours is attended in most cases by marked retardation of the E.S.R., making the test valueless after this time. Our results are contrary to those of Proescher (1951), who found that the "sedimentation speed remained steady for several days" if the blood was stored at 5° C., the E.S.R. being estimated by the Wintrobe technique. We regard it as wise practice to discard samples of sequestrene blood after 24 hours' refrigerated storage, at least so far as the E.S.R. is concerned.

Storage of sequestrene blood for six hours at room temperature does not affect the E.S.R. appreciably, but storage for a 24-hour period is usually followed by

marked retardation of the E.S.R. A six-hour period in which the blood can stand on the side-room or laboratory bench gives ample time for an accurate estimation of the E.S.R. Advantage can be taken of this by workers in haematology departments who have many samples delivered to them in a short space of time, and also by general practitioners who may wish to take blood during a busy surgery.

The hypothesis that because the E.S.R. at six hours is the same as the immediate E.S.R. of any sample, then at any intervening time the E.S.R. will be similar, is borne out by the small series of tests in experiment 4. The intermittent mixing of the same sample, as shown by experiment 5, has no apparent effect on the E.S.R., which probably slows after about eight hours' storage. It is probably safe to assume that such mixing as a sample might receive in a practitioner's case, car, or pocket will have no effect on the E.S.R. within the six-hour period following withdrawal of the blood. There must, of course, be adequate mixing of the blood sample at the time of setting up the E.S.R.

The use of sequestrene blood thus enables E.S.R.s to be estimated at any time up to six hours after withdrawal of the blood, and extends the usefulness of this clinical test.

Summary

The need to set up E.S.R. estimations immediately after withdrawal of blood samples is obviated by taking the blood into the anticoagulant sequestrene. When this is used there is no significant retardation of the E.S.R. up to six hours after the blood is taken. It does not matter whether the blood stands undisturbed or is mixed at intervals, provided that it is thoroughly mixed before the estimation is made. Storage for longer periods at room temperature is often accompanied by marked retardation of the E.S.R.

Storage of sequestrene blood in the refrigerator for 24 hours does not affect the E.S.R., but storage for 48 hours is usually attended by marked slowing of the E.S.R. It is recommended that sequestrene blood should not be used for E.S.R. estimations if it has been kept in the refrigerator beyond 24 hours.

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A year ago the Council of Europe concluded an agreement whereby the signatory countries undertook to grant each other assistance with regard to the supply of blood and blood products, thereby taking the first steps towards setting up what virtually amounts to a European blood bank. The Council's Committee of Experts on Public Health, at a recent meeting in Strasbourg, examined the possibility of concluding another similar agreement providing for the pooling of test sera of human and other origin for purposes of blood-group determination. A preliminary study of this question was undertaken in July, 1959, in London by a group of doctors who will again meet this March. A draft European agreement on the exchange of test sera will be prepared for the consideration of the Council's Public Health Committee at its next session in June.

Preliminary Communications

Some Experimental and Clinical Data on the Sarcylisin Peptides

Since 1956 the synthesis and study of peptides and amides of sarcylisin have been carried out at the Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the U.S.S.R., in collaboration with the Institute of Element-organic Compounds, on the presumption that the use of the carrier of chloroethylamino groups may result in the production of compounds with a varying effect upon tumour spectra (Larionov, 1956, 1957, 1958b, 1959a, 1959b).

The first peptides of N-formyl-sarcylisin were synthesized by Knunianz, Kildisheva, and Golubeva (1956). Experimental study of these compounds carried out by Larionov and Sophina (1957) and Larionov (1958a) showed first of all that these are but slightly toxic. Thus the LD₁₀₀ for mice amounts to 750–1,000 mg./kg. Nevertheless, all these compounds showed anti-tumour activity in mice and rats at a dose of 25–60 mg./kg. daily by intraperitoneal injection and of 200–300 mg./kg. by mouth. It was also found that these peptides vary in their action upon the tumour spectrum in mice and rats. In addition, sarcylisin peptides were found to inhibit haemopoiesis to a lesser degree than does sarcylisin itself.

Subsequently, peptides of N-acetyl-sarcylisin have been synthesized (E. N. Shkodinskaya and others). Experiments on animals (Sophina, 1959) confirmed the data obtained with peptides of N-formyl-sarcylisin, with the difference that some of them, such as N-acetyl-sarcylisil-valine ethyl ester ("asaline"), proved effective at smaller doses. Their therapeutic intraperitoneal dose for rats is 7–25 mg./kg. daily.

Each of the N-acetyl peptides likewise has its own "action spectrum" with regard to animal tumours. Thus asaline cures rat sarcoma 45, inhibits growth of sarcoma M-I and 298 by 98%, but sarcoma 37 by only 50%. N-acetyl-sarcylisil-methionine ethyl ester likewise cures sarcoma 45 almost as effectively, but is much less effective with regard to sarcoma M-I and 298 (88% and 30% inhibition), though it is more effective than asaline with regard to sarcoma 37 (88% inhibition).

Clinical tests at the Research Hospital of the Institute of Experimental and Clinical Oncology, under the guidance of N. N. Blokhin, have as yet been carried out only with asaline (19 patients). Daily doses of 1 to 2 g. have been used, but it is not yet certain whether these are optimal doses. Total doses amounted usually to about 20 g.

The Table shows the number of patients, kind of tumour, and the effect of treatment.

The therapeutic effect manifested itself in partial, and less often in complete, regression of the tumours. In two patients with seminoma metastases and in three with myelomatosis relapses were recorded within one to three months. A positive effect was obtained after repeated courses of asaline treatment of myelomatosis. Both patients with far advanced