I. J. Med. Sc.
Seventh Series. Vol. I. No. 5
May, 1968

PHYSIOLOGY OF FIBRINOLYSIS*

III. *The effect of exercise on fibrinolysis in health and Multiple Sclerosis.*

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Summary

THE effect of exercise on the dilute blood clot lysis time (B.L.T.), plasma fibrinogen, and serum anti plasmin has been investigated in two groups of healthy volunteers and in patients with multiple sclerosis.

The first group of normal volunteers were studied over a two hour period at rest and following exercise. The second group was investigated immediately before and five minutes after exercise. The patients with multiple sclerosis were similarly investigated.

The mean B.L.T. is longer in multiple sclerosis than in normal subjects. There is significant shortening of the B.L.T. and a rise, which does not reach significance, of the antiplasmins in both normal subjects and patients with multiple sclerosis, following exercise. The fibrinogen, which falls in normal subjects after exercise, rises in multiple sclerosis.

The importance of investigation of the fibrinolytic system over a period of time or following stimulation is stressed.

Introduction

It is well recognised that exercise serves as a physiological stimulus to the fibrinolytic system (Biggs *et al.*, 1947; Truelove, 1951; Fearnley and Lackner 1955; Sherry *et al.*, 1959; Billimoria *et al.*, 1959; Ratnoff and Donaldson, 1960; Jang *et al.*, 1964; Ogston and McAndrew, 1964; Cash, 1966; Menon *et al.*, 1967).

It has been postulated that microthrombii, composed mainly of platelets are responsible for the plaques in multiple sclerosis (Persson, 1958), and it has been claimed that exercise is of benefit in the treatment of multiple sclerosis (Russell, 1966). The response of the fibrinolytic system to stimulation by exercise might, therefore be expected to be of importance in this disease.

Materials

Plasmin: Kabi 1398, human plasmin grade "b" measured in casein units (Sgouris) Lot. No. LmD3.

Bovine Fibrinogen: Kabi Grade "B1" Lot No. Qdx 19, 0·2% stored as 0·6% in 2·8% NaCl 1 at −20° C, thawed and diluted 1/3 with Tris Buffer, pH 7·4.

*Note.—No. I of the present series was published in this JOURNAL in October, 1966, and No. II in February, 1967.

Thrombin:	Thrombin, topical (bovine origin) Parke Davis & Co. 100 units/ml. in Tris Buffer at pH 7·4 stored at −20° C.
Reference Serum:	Pooled sera from 20 healthy humans stored at −20° C and diluted 1/8 in saline at time of use.
Normal Saline:	0·9% NaCl. (Clinical) Antigen (Ireland) Ltd.
Syringes:	Plastic, disposable.

Methods

Subjects

Normal volunteers: In the first study, 3 male volunteers, between the ages of 20 and 30 years were selected. In the second study, 20 volunteers (15 male, 5 female) between the ages of 18 and 60 years were selected. Multiple sclerosis patients: A total of 26 patients (18 female, 8 male) between the ages of 16 and 63 with multiple sclerosis were investigated. All cases had been diagnosed as definitely suffering from multiple sclerosis and any cases considered to be doubtful were excluded.

Exercise

A "Lode" ergometer was used so that the amount of exercise could be measured and it was found that the following amounts of exercise produced moderate dyspnoea and caused perspiration in the majority of normal volunteers studied and it was considered likely that fibrinolysis would be stimulated at these values:

Males	150 watts for 5 minutes
Females	100 watts for 5 minutes.

The volunteers were able to comply with this requirement but nearly all were pleased when the exercise was completed.

The amount of exercise was not initially standardised in the multiple sclerosis patients, the patient being instructed to exercise as much as possible. It later became apparent that in many instances the patient was capable of completing a normal amount of exercise and thereafter was exercised according to the above standards. If the disease affected the patient so that the legs could not be used for exercise, the ergometer was adjusted and exercise was performed with the arms.

The affect of exercise was studied in two stages in the normal subjects.

Stage I

In the first stage blood samples were withdrawn at fifteen minute intervals over a period of two hours from three subjects.

Prior to the experiment the subject was instructed to refrain from smoking and to have a light breakfast. The experiment was commenced each day at 9.30 a.m. and was conducted over five days, with at least one day interval between each experiment day.

To facilitate the collection of blood and to avoid repeated venepunctures a saline infusion was erected at the start of the experiment. The infusion was run slowly throughout the morning.

The experiment plan was as follows:

Day 1. Basal day: Subject lies on a bed reading in a quiet room for 2 hours. Blood samples were withdrawn at 15 minute intervals.

Day 2. Exercise day: Subject lies on a bed for one hour during which time blood samples are withdrawn every 15 minutes. At the end of one hour he is exercised for 5 minutes according to the standard set out above and a blood sample is taken at the completion of exercise. The subject returns to bed for a further hour during which time blood samples are withdrawn at 15 minute intervals.

Day 3. Basal day.

Day 4. Exercise day.

Day 5. Basal day.

Stage II

In the second stage blood samples were withdrawn immediately before and five minutes after completion of exercise in 20 normal subjects. In some cases exercise was repeated giving a total of 37 estimations. A similar programme was conducted in 26 patients with multiple sclerosis. Exercise was repeated in a number of instances bringing the total number of estimations to 68.

Collection of Blood

All blood samples were obtained in plastic syringes from an antecubital vein and the use of a tourniquet was avoided where possible. An aseptic technique was employed in all cases.

The blood was transferred immediately to the appropriate container in melting ice and throughout the tests all blood samples were kept in an ice bath.

Tests

The following tests were done, except where otherwise stated on each blood sample:

Dilute Blood Clot Lysis Time (B.L.T.): This was done in duplicate by the method of Fearnley, Balmforth and Fearnley (1957). The end point was modified as described by Fearnley & Chakrabarti (1962). The lysis times are inversely related to the fibrinolytic response and the normal range for this test is 3 to 9 hours.

Serum Antiplasmin Activity: The method of Guest, Ware and Seegers (1947) as modified by Thornes, O'Donnell and O'Brien (1967) was employed. This test is a measure of total antiplasmin activity of the serum. The normal range is 90% to 180%.

Fibrinogen Levels: The fibrinogen levels were estimated by the method of Blombäck and Blombäck (1956). The normal range is 100 to 300 mgms. per cent.

Statistical Methods

The mean percentage changes were calculated for each study group. Comparison of the means were made by the Students t Test and the significance level was taken to be the 5% probability. Snedecor's F Ratio Test was used to test the variance of the different groups.

Results

Stage 1. Estimations at fifteen minute intervals over a two hour period under basal conditions and following exercise.

The behaviour of the means of the three parameters under basal conditions and following exercise is graphically depicted in Fig. 1.

The B. L. T. is significantly shortened after the completion of exercise and has returned to pre-exercise levels within an hour but remains unaltered under basal conditions.

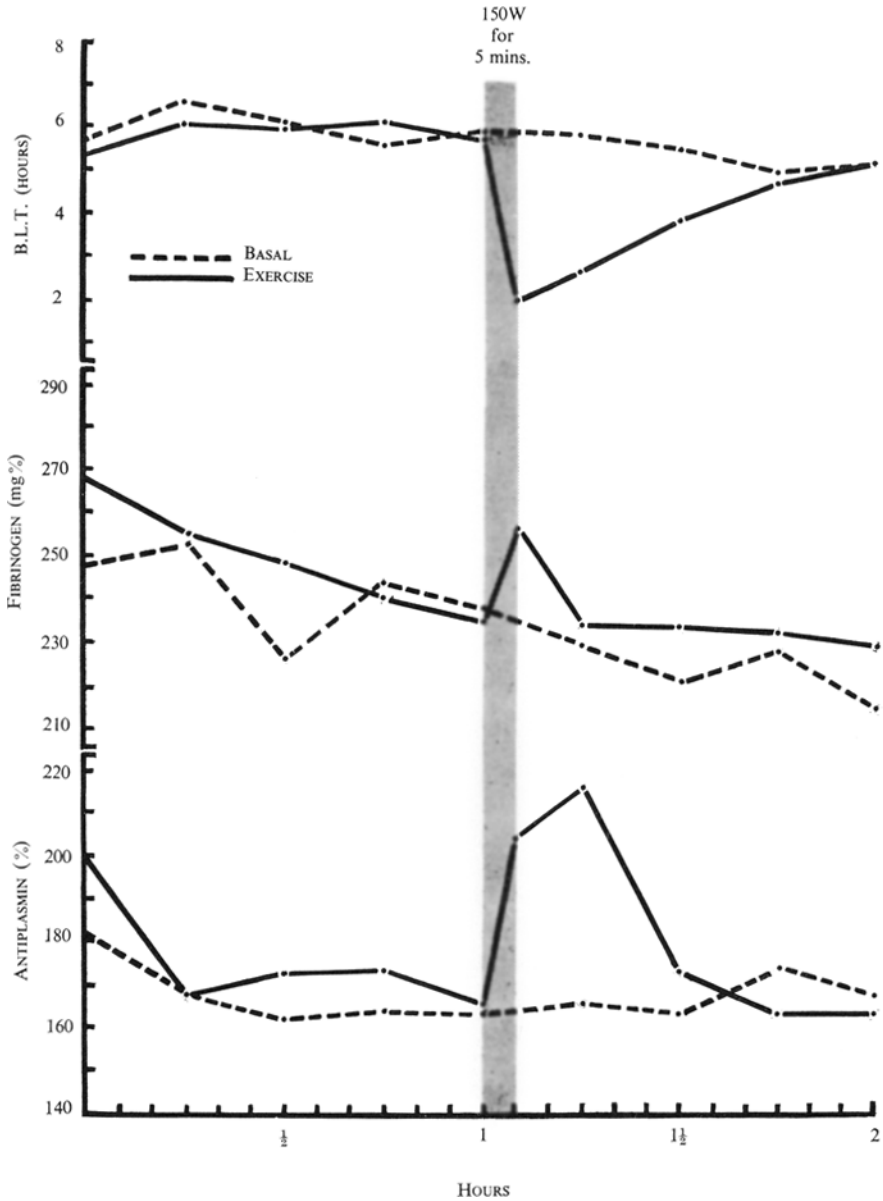

Fig. 1. Means of serial estimations of antiplasmin, fibrinogen and B.L.T. in normal subjects during rest (9 experiments) and before and after exercise (6 experiments).

The fibrinogen level rises immediately after exercise but not to significant proportions. Under basal conditions there is a slight but gradual fall in fibrinogen levels.

The antiplasmins rise immediately after exercise, reaching a maximum 10 minutes after the completion of exercise and have returned to pre-exercise levels after 40 minutes. This rise is not statistically significant in the group studied. Under basal conditions this parameter remains constant.

Stage II. Estimations immediately before and five minutes after the completion of a standardised amount of exercise.

The mean values for the three parameters studied are shown before and after exercise in Table I. The mean antiplasmin level before exercise was 157%. The mean B.L.T. before exercise was 7.27 hours, and the mean fibrinogen level before exercise was 248 mgms%. Only in the B.L.T. was there a statistically significant fall in the arithmetical mean following exercise. The mean percentage changes after exercise are shown in Table 1. There is a significant mean percentage fall in the fibrinogen levels after exercise and the B.L.T. is significantly shortened following exercise. There is no significant percentage alteration in the antiplasmin levels after exercise.

TABLE 1

Effect of Exercise on Antiplasmin, B.L.T. and Plasma Fibrinogen in Normal Subjects and Patients with Multiple Sclerosis.

	Normal			Multiple Sclerosis		
	Anti-plasmin	B.L.T.	Fibrinogen	Anti-plasmin	B.L.T.	Fibrinogen
Mean values						
Before exercise	157%	7·27 hrs.	248mgm.%	188%	15·2 hrs.	236mgm.%
After exercise	162%	5·45 hrs.	240mgm.%	214%	12·9 hrs.	277mgm.%
Probability	37%	2·8%	32%	98%	21%	31%
Mean percentage change after exercise*	+3·8%	−19·8%	−25·7%	+7·2%	−14·7%	+7·1%
Probability	25%	2%	>0·01%	7%	>0·1%	19%
Number of Estimations	37	32	27	68	65	18

*Plus indicates a rise; Minus indicates a fall.

Multiple Sclerosis

The mean values before and after exercise are shown in Table 1. The mean antiplasmin level before exercise was 188%. The mean B.L.T. was 15.2 hours and the mean fibrinogen level was 236 mgms.% before exercise. The B.L.T. is significantly longer in multiple sclerosis than in the normal subject.

The mean percentage changes after exercise are shown in Table I. No

significant change occurs in the antiplasmin levels. There is a significant shortening of the lysis time following exercise. The plasma fibrinogen rises but does not reach significant proportions.

Age and Sex

There was no correlation between the fibrinolytic response after exercise and age in multiple sclerosis. In the normal group, the response to exercise was less over the age of 30 years. This was statistically significant with regard to the antiplasmin levels.

There was no significant difference between the sexes.

Discussion

In normal subjects studied serially over a two hour period, increased fibrinolytic activity as reflected by the B.L.T., has been shown to occur after exercise. This activity was maximal at the completion of exercise and had returned to pre-exercise values at the end of one hour (Fig. 1.) Under resting conditions the B.L.T. did not alter. The persistence of an enhanced fibrinolytic state following exercise has been reported as varying from 30 minutes to over 3 hours. (Biggs *et al.*, 1947; Sherry *et al.*, 1959; Billimoria *et al.*, 1959; Ogston and Fullerton, 1961; Menon *et al.*, 1967). The variation in the duration of the fibrinolytic state following exercise probably depends on the severity and duration of the exercise as well as on other variables such as age and physical fitness. A rise in antiplasmin occurred in these subjects. This was maximal 10 minutes after the completion of exercise and had returned to pre-exercise levels 40 minutes later. The antiplasmins did not vary under resting conditions. The period of increased inhibition coincides with the period of enhanced fibrinolytic activity. This would suggest a state of dynamic equilibrium between the activator and inhibitor systems. The increase in fibrinolytic activity following exercise has been shown to be due to increased production of plasminogen activator, but it has not been possible to demonstrate increased levels of circulating plasmin following exercise and the level of antiplasmin has not previously been shown to alter after exercise (Sherry *et al.*, 1959; Sawyer *et al.*, 1960). It is apparent that the alteration in inhibitor level is of brief duration and sample timing is obviously of importance in investigating any parameter of the fibrinolytic system. A gradual fall in fibrinogen occurred over a two hour period under resting conditions (Fig. 1.). This tendency was also apparent during the resting phase prior to exercise, and a small transient rise in fibrinogen followed exercise. Previous workers have not demonstrated any alteration in the fibrinogen during periods of increased fibrinolytic activity (Sherry *et al.*, 1959; Sawyer *et al.*, 1960; Ratnoff and Donaldson, 1960).

Following on the above observations a further 20 normal volunteers were exercised and blood samples were withdrawn immediately before and five minutes after the completion of exercise. The B.L.T. was, again, significantly shortened after exercise. Antiplasmin levels rise but not significantly, and the fibrinogen levels show a significant mean percentage fall in contrast to the subjects studied over a two hour period. These conflicting results may be accounted for by the gradual fall which occurs in this parameter

during rest; the subjects studied five minutes after exercise had not rested prior to the procedure.

In the patients with multiple sclerosis the mean B.L.T. is twice as long as in the normal subjects, and following exercise there is significant shortening of the lysis time. The antiplasmins rise more than in the nomal subjects but do not reach significance. There is, therefore, adequate activation of fibrinolysis without excessive inhibition, following exercise. In contrast to the normal subjects investigated similarly, a rise occurs in the fibrinogen levels following exercise. The multiple sclerosis patients were by virtue of their disease, unaccustomed to physical activity and had arrived directly from the wards or by car for the examination. In this respect, they resembled the normal subjects who had rested for an hour prior to exercise, whereas the second group of volunteers had been actively engaged in their work before coming to the laboratory. The rise in fibrinogen after exercise in the patients with multiple sclerosis may therefore be similar, although much greater, to that occurring in the resting volunteers.

Statistical comparison between the normal subjects and the multiple sclerosis patients before and after exercise showed that the variances differed significantly ($P<5\%$), indicating that the two samples came from different populations. This was most marked in comparison of the fibrinogen levels.

Rindfleisch in 1863 observed that the plaques in multiple sclerosis are commonly centered on small blood vessels. Putnam (1937) has suggested that the plaque results from microthrombii in the central venule, and Persson (1958) has proposed that platelets are responsible for these microthrombii. Nathanson and Savitsky (1954) were the first to study platelet adhesiveness in multiple sclerosis and they demonstrated that this is increased during the active phase of the disease. This has since been confirmed (Caspery *et al.*, 1963; Wright *et al.*, 1965). In 1964, Field and Caspery showed that the addition of an encephalitogenic factor isolated from human brain caused increased platelet stickiness in active phases of multiple sclerosis, but not in healthy persons and only to a slight extent in patients in the quiescant phase. It would appear, therefore, that the disorder which results in the plaque in multiple sclerosis is of vascular rather than neural origin.

Russell (1966) considered that exercise would possibly influence an intermittent circulatory abnormality, by leading to a physiological hyperaemia of the spinal cord segments principally involved in the exercises. He commenced a rigorous athletic training programme for his cases of multiple sclerosis and suggests that this was of considerable benefit.

The fibrinolytic abnormalities in multiple sclerosis may be associated with the abnormal platelet adhesiveness in this disease or these abnormalities might be accentuated during active phases of the disease, as is platelet adhesiveness. Constant stimulation of fibrinolysis, by a rigorous exercise programme, might then be of considerable benefit to the patient.

The fibrinolytic system is considered to be in a state of dynamic equilibrium within itself and with the coagulation mechanism (Astrup, 1956). Knowledge of fibrinolysis will be advanced by investigation of this system whilst it is functioning. This is best achieved by serial observations over a period of time or following a suitable stimulus. Erroneous conclusions could be arrived at if too much credence is attached to isolated estimations.

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Acknowledgements

This study was supported by the Medical Research Council of Ireland, the Irish Cancer Society and Tenovus.

We wish to thank the physicians of St. Laurence's Hospital for permission to examine patients under their care, Dr. E. Martin, Adelaide Hospital for advice and guidance in organising the study, Dr. T. M. Gregg and Miss E. Pearson for facilities and assistance in the Physiotherapy Department, Miss H. Glanville for secretarial assistance, Mr. K. Wilson for statistical advice, and Mr. B. Hogan for technical help.

We are grateful to Kabi.AB. Sweden for donations of Plasmin and fibrinogen.

Requests for reprints should be addressed to R.D.T.