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# EFFECT OF EXERCISE ON THE LEVEL OF ANTIHAEMOPHILIC GLOBULIN IN HUMAN BLOOD

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It is thought that the coagulation defect in haemophilia is due to the absence of an essential blood-clotting factor. This factor, called antihaemophilic globulin (AHG), is present in fresh normal blood, but deteriorates when blood is stored. At present the only way of correcting the haemostatic and coagulation defect in haemophilia is by transfusions of blood, plasma or plasma protein concentrates rich in antihaemophilic globulin, and it is therefore desirable to understand the mechanism whereby AHG activity disappears from stored blood and plasma.

The fibrinolytic enzyme, plasmin, is one of the agents which is known to destroy AHG (Wagner, Pate & Brinkhous, 1954; Brakman, van Creveld, Engelsman, van't Laar, Mochtar & Veder, 1959) and Penick & Brinkhous (1956) have suggested that the loss of AHG activity of blood on storage might be due to the presence of traces of plasmin.

Biggs, Macfarlane & Pilling (1947) demonstrated increased fibrinolytic activity in the blood of normal people after vigorous exercise. This observation seemed to provide a satisfactory way of studying the effect of the naturally occurring fibrinolytic enzyme on the stability of AHG in stored blood, and experiments were done in which the deterioration of AHG was studied in the blood collected from healthy subjects before and after exercise. In the course of the investigation it was observed that the blood samples taken after exercise showed much more AHG activity than the samples taken before exercise. This unexpected finding is the subject of this paper.

## METHODS

Blood was collected by venepuncture into a silicone-coated syringe and mixed with 1/9 volume of trisodium citrate (3·8 g/100 ml.) in a silicone-coated tube. The blood was then spun at 700 *g* for 10 min and the supernatant plasma separated.

*Assay of AHG* was performed according to the method of Biggs, Eveling & Richards (1955). The AHG level of the blood after exercise was expressed as a percentage of the level before exercise. In the experiments in which plasma was infused into haemophiliacs the AHG levels were expressed as a percentage of a standard pool of four normal plasma samples.

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*Factor-V-deficient plasma* was prepared by incubating oxalated plasma at 37° C for 36 hr.

*Factor V* was assayed by determining the ability of the test plasma to correct the prolonged one-stage prothrombin time of Factor-V-deficient plasma (Owren, 1947).

*Fibrinogen* concentration of plasma was estimated by clotting the diluted plasma with 0·025 M-$CaCl_2$ and then carrying out a standard micro-Kjeldahl digestion on the washed clot.

*Prothrombin* was measured by the two-stage method of Biggs & Douglas (1953*a*).

*Christmas factor* was assayed according to the method of Biggs, Bidwell, Handley, Macfarlane, Trueta, Elliot-Smith, Dike & Ash (1961). This assay is based on the thromboplastin-generation test of Biggs & Douglas (1953*b*) and measures the ability of a test serum to correct the defective thromboplastin generation in the serum of a patient suffering from Christmas disease.

*The one-stage prothrombin time* was determined by a modification of Quick's one-stage procedure as described by Biggs & Macfarlane (1957).

*The whole-blood clotting time* and *the plasma recalcification time* in glass and silicone-coated tubes were carried out as described by Biggs & Macfarlane (1957).

*The thromboplastin-generation test* was carried out as described by Biggs & Douglas (1953*b*), except that a chloroform extract of brain was used in place of a platelet suspension.

*The prothrombin consumption test* was performed according to the method of Merskey (1950) and expressed as an index.

*Fibrinolytic activity* in citrated plasma was demonstrated by the method of Macfarlane & Biggs (1946).

*Exercise procedure.* All the subjects performed the same exercise, which entailed running as fast as possible around the hospital perimeter, a distance of approximately ¾ mile (1·2 km). The time taken to complete the run was 5–8 min and varied with the physical fitness and age of the subject. Blood samples were taken by venepuncture immediately before and immediately after exercise and in some cases at varying intervals throughout the day.

## RESULTS

### *Effect of exercise on AHG activity in the blood of normal subjects*

The effect of exercise on the level of AHG activity in the blood of normal people was studied in 18 experiments on fifteen subjects (eleven males, four females, aged 18–55 years). In each case there was a marked increase in the AHG activity of the blood after exercise, irrespective of the age or sex of the subject (Table 1).

Additional blood samples were obtained from four of the above subjects at intervals of 2, 4 and 6 hr after exercise, in order to see how long the level of AHG activity would remain raised. The average AHG level of blood taken from four subjects 6 hr after exercise was still about 60% higher than the control level. There was considerable variation from person to person in the percentage increase of AHG after exercise and the response of the same individual varied from day to day, as is shown by the figures for subjects 2 and 5 in Table 1.

In the above experiments the exercise performed was strenuous. The effect of less vigorous exercise on the AHG level was studied in five normal subjects after they had walked 3 miles (4·8 Km) and 6 miles at an average speed of 5 miles/hr. This amount of exercise failed to raise the AHG level.

After completing the 6 mile walk, two of the subjects ran ¾ mile as fast as possible. The level of AHG in both cases rose to 200 % of the level before exercise.

### *Effect of repeated venepuncture on AHG activity in the blood of normal subjects*

To investigate the possibility that the trauma and pain of venepuncture might in some way raise the level of AHG activity, blood samples were taken at 10 min intervals from six healthy volunteers (three males, three females) at rest. Four of the subjects had three venepunctures and the

TABLE 1. Effect of exercise on plasma AHG level of normal subjects

| Subject | Age | Sex | Level of AHG after exercise (% of level before exercise) |
|---|---|---|---|
| 1 | 28 | *M* | 200 |
| *2 | 30 | *M* | (*a*) 143 |
| | | | (*b*) 200 |
| 3 | 30 | *M* | 314 |
| 4 | 24 | *F* | 180 |
| *5 | 28 | *M* | (*a*) 180 |
| | | | (*b*) 227 |
| | | | (*c*) 300 |
| 6 | 18 | *F* | 200 |
| 7 | 25 | *M* | 333 |
| 8 | 25 | *M* | 250 |
| 9 | 23 | *F* | 231 |
| 10 | 21 | *F* | 170 |
| 11 | 55 | *M* | 260 |
| 12 | 35 | *M* | 200 |
| 13 | 30 | *M* | 200 |
| 14 | 28 | *M* | 215 |
| 15 | 23 | *M* | 139 |
| | | | Average 219 |

* The figures *a*, *b* and *c* were obtained on subjects 2 and 5 on different days.

remainder had two each. There was no consistent change in the AHG level with successive venepunctures, the average levels being 101 % after the second venepuncture and 97 % after the third venepuncture compared with an initial level of 100 %. All the subjects were accustomed to venepuncture and in previous experiments all had shown a rise of AHG after exercise.

### *Effect of exercise on the level of AHG activity in the blood of mildly affected haemophilic subjects*

It having been observed that strenuous exercise raises the AHG activity of the blood of normal subjects, experiments were carried out to see if haemophilic subjects would react to exercise in a similar manner. Only mildly affected haemophiliacs took part in these experiments, the risk of joint and muscle injury in severely affected haemophiliacs being too great to justify their taking part in such an investigation.

Three experiments were carried out on two haemophilic volunteers. On the basis of family history, severity of bleeding and level of AHG in their blood, both were considered to be mild cases (Biggs & Macfarlane, 1958). Samples of blood were taken before and after a $\frac{3}{4}$ mile run and the level of AHG activity assayed. In each case there was a marked increase in the AHG activity after exercise, the level after exercise being approximately 250 % of the level before (Table 2). The increase in activity is thus of the same order as that obtained in the normal subjects.

TABLE 2. Effect of exercise on the plasma AHG level of mildly affected haemophilic subjects

| Subject | Age | AHG level (% of normal plasma) Before exercise | After exercise | AHG level after exercise (% of level before exercise) |
|---|---|---|---|---|
| A.T. | 22 | (*a*) 25 | 60 | 240 |
| | | (*b*) 30 | 70 | 233 |
| J.B. | 26 | 15 | 40 | 266 |
| | | | | Average 246 |

Lines (*a*) and (*b*) refer to two runs by A.T. on different days.

### *Possibility of artifact*

*Effect of exercise on levels of other clotting factors.* It is possible that the increase of AHG in the blood recorded after exercise is an artifact and is due to the assay system being sensitive to changes in some other plasma constituent. In the assay method used in this study the test plasma was adsorbed with $Al(OH)_3$ and then various dilutions of the plasma were tested in a modified thromboplastin generation test. It has been found in this test that the amount of thromboplastin generated is directly proportional to the amount of AHG present, provided the other clotting factors are kept constant. It was therefore essential to study the level of other clotting factors after exercise, especially those factors known to take part in the generation of thromboplastin. In experiments on two normal subjects the levels of Christmas factor, Factor V, prothrombin, fibrinogen and AHG were assayed before and after exercise. The level of AHG activity rose in both subjects, but there was no increase in any of the other factors studied (Table 3).

*Possible effect of fibrinolysin on the AHG assay.* Biggs *et al.* (1947) have shown that strenuous exercise produces fibrinolytic activity in the blood of normal human beings. In the present study all the subjects who undertook vigorous exercise showed marked fibrinolytic activity in their blood as well as a rise in AHG activity, whereas those subjects who performed less vigorous exercise, a 3 mile and 6 mile walk, for instance, showed little or no increase in fibrinolytic or AHG activity. These findings suggested that the presence of fibrinolysin in the blood might be affecting the AHG

assay and giving falsely high results. This possibility seemed unlikely, however, when it was found that blood collected 4 and 6 hr after exercise still showed high AHG levels (see p. 129) but no detectable fibrinolytic activity. Moreover, in experiments in which streptokinase was added to fresh citrated blood to activate the fibrinolytic system, no rise in AHG activity could be demonstrated. On the contrary, there was a loss of AHG activity.

*Infusion into haemophiliacs of plasma from exercised donors.* If plasma collected after exercise contains 2–3 times the normal amount of AHG one would expect that an infusion of this plasma into a haemophiliac would produce a greater rise in his blood AHG level than would an infusion of plasma from non-exercised donors. Two severely affected haemophiliacs,

TABLE 3. Effect of exercise on level of AHG and other clotting factors in the plasma of two normal subjects

| | Level after exercise (% of level before exercise) | |
|---|---|---|
| | Subject 1 | Subject 2 |
| AHG | 275 | 250 |
| Factor V | 100 | 100 |
| Prothrombin | 105 | 108 |
| Christmas factor | 70 | 100 |
| Fibrinogen | 108 | 108 |

F.E. and R.B., were each given 1 litre of plasma prepared from the blood of donors who had performed strenuous exercise. Samples of blood were taken from the two subjects immediately before and immediately after the infusion and then 24 hr later. The AHG response of patient F.E. was consistent with his plasma dose containing 200 % of AHG, but in the case of R.B. the response was more than would have been expected from plasma in the dose which he was given. This is so far unexplained. The blood of both patients contained 10 % of AHG 24 hr after the infusion (Table 4).

Three days before the above experiment was carried out R.B. was given 1 l. of plasma collected from non-exercised donors. The AHG content of this plasma dose was 80 % of normal plasma and the patient's AHG level immediately after the infusion had risen to 12 % of normal. Twenty-four hours later the level had returned to zero.

Strictly speaking, these experiments served mainly as a check on the AHG level of the administered plasma. The best evidence for increase in AHG activity would be the demonstration that plasma from exercised donors was more effective than plasma from non-exercised donors in controlling haemophilic bleeding. It would be difficult, however, without an extensive clinical trial to differentiate between plasma doses containing 100 and 200 % of AHG respectively.

*Effect of exercise on other tests of blood clotting*

Several workers have found that the clotting time of blood is shortened by exercise (Hartman, 1927; Mills & Necheles, 1928; Schneider & Zangari, 1951). In view of these reports of increased coagulability of the blood after exercise, experiments were done in which the AHG assay was carried out in parallel with some routinely used tests of clotting function. Five normal subjects and two mildly affected haemophiliacs took part in this investigation. The tests carried out in addition to the AHG assay were: the whole blood clotting time in glass and siliconed tubes, the recalcification time of platelet-rich and platelet-poor plasma in glass and siliconed tubes, the Quick one-stage test and the thromboplastin-generation test. In the case of the two haemophiliacs the prothrombin consumption index was also determined. In none of the tests on normal subjects did the blood or plasma consistently show more rapid clotting after exercise, although the

TABLE 4. AHG response of haemophilic subjects following infusions of plasma from exercised and non-exercised donors

| | AHG levels (expressed as % of normal plasma) | | |
|---|---|---|---|
| | Plasma dose prepared from blood of exercised donors | | Plasma dose prepared from blood of non-exercised donors |
| | R.B. | F.E. | R.B. |
| AHG activity in patients' blood before dose | 0 | 0 | 0 |
| AHG activity of dose | 152 | 200 | 80 |
| AHG activity in patients' blood after dose | 50 | 25 | 12 |
| AHG activity in patients' blood 24 hr after dose | 10 | 10 | 0 |

level of AHG activity in every case rose to more than 200 % of the level before exercise. With regard to the tests in the two haemophilic subjects, the whole-blood clotting time, recalcification time and thromboplastin-generation test were normal before exercise, and showed no change after exercise, but the prothrombin consumption index in both was found to be slightly abnormal before exercise, 23·6 and 16 %, and normal after exercise, 9·2 and 7·1 %, respectively. (In this laboratory, at present, the upper limit of 'normal' for the prothrombin consumption index is 10 %.)

## DISCUSSION

In the past the effect of exercise on the blood-clotting mechanism has received little attention. The present investigation shows that strenuous muscular exercise brings about a marked increase of AHG activity in the blood of normal subjects and mildly affected haemophiliacs.

Hartman (1927) found that cat's blood clotted more quickly after exercise and Mills & Necheles (1928), experimenting on human beings and

dogs, also found more rapid clotting after exercise. More recently Schneider & Zangari (1951) have shown that the clotting time of blood in silicone-coated tubes is accelerated after exercise. In the light of the present study it seemed possible that the raised coagulability of the blood after exercise observed by these workers might be related to the increase of AHG activity. In a series of experiments carried out to investigate this possibility it was found that even with an increase of AHG to 250 % of normal there was no convincing evidence that blood clotted more rapidly after exercise.

The finding that the AHG level is increased by exercise is of practical and theoretical importance. Several workers consider that the AHG content of the blood is genetically determined and fixed for the individual (Graham, McLendon & Brinkhous, 1953; Brinkhous, Langdell, Penick, Graham & Wagner, 1954). Although it is possibly true that each individual has a basal level of AHG which is genetically determined, there seems little doubt from the above experiments that the level is not fixed but is labile. This is of considerable practical importance with regard to the AHG assay. In this assay, as in other biological assays involving impure systems, the provision of a suitable standard presents a problem. Biggs (1957) used a pool of four plasma samples to represent 100 % of AHG, whereas others (Pool & Robinson, 1959) used plasma samples obtained from the same individuals at intervals and stored at $-20°$C. Biggs *et al.* (1955) used a freeze-dried concentrate of bovine AHG as a standard and this, in view of the effect of exercise in the level of AHG, would seem to be the most satisfactory standard, especially when one wishes to compare the AHG levels of individuals from day to day.

The mechanism whereby exercise produces a rise in the AHG activity of the blood is not known, but further study of this phenomenon may lead to the understanding of the conditions which govern the level of AHG in the blood of normal people and may throw more light on the nature of the coagulation defect in haemophilia.

## SUMMARY

1. Strenuous physical exercise increased the AHG activity of the blood of normal people and of mildly affected haemophiliacs. The rise in AHG activity was apparent after 4–6 min of vigorous exertion and persisted for at least 6 hr.

2. There was no increase in the levels of Christmas factor, Factor V, prothrombin or fibrinogen with exercise.

3. Plasma from exercised donors was infused into two severely affected haemophiliacs. The AHG response in the blood of the haemophilic sub-

jects confirmed that plasma after exercise contained increased AHG activity as reflected in the assay.

4. The high AHG level after exercise was not associated with increased coagulability of the blood in the routinely used blood clotting tests.

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## REFERENCES

BIGGS, R. (1957). Assay of antihaemophilic globulin in treatment of haemophilic patients. *Lancet*, ii, **273**, 311–314.

BIGGS, R., BIDWELL, E., HANDLEY, D. A., MACFARLANE, R. G., TRUETA, J., ELLIOT-SMITH, A., DIKE, G. W. R., ASH, B. J. (1961). The preparation and assay of a Christmas factor (factor IX) concentrate and its use in the treatment of two patients. *Brit. J. Haemat.* (in the Press).

BIGGS, R. & DOUGLAS, A. S. (1953*a*). The measurement of prothrombin in plasma. *J. clin. Path.* **6**, 15–22.

BIGGS, R. & DOUGLAS, A. S. (1953*b*). The thromboplastin generation test. *J. clin. Path.* **6**, 23–29.

BIGGS, R., EVELING, J. & RICHARDS, G. (1955). The assay of antihaemophilic globulin activity. *Brit. J. Haemat.* **1**, 20–34.

BIGGS, R. & MACFARLANE, R. G. (1957). *Human Blood Coagulation and its Disorders*, 2nd ed. Oxford: Blackwell Scientific Publications.

BIGGS, R. & MACFARLANE, R. G. (1958). Haemophilia and related conditions: a survey of 187 cases. *Brit. J. Haemat.* **4**, 1–27.

BIGGS, R., MACFARLANE, R. G. & PILLING, J. (1947). Observations on fibrinolysis. Experimental activity produced by exercise or adrenaline. *Lancet*, **252**, 402–411.

BRAKMAN, P., VAN CREVELD, S., ENGELSMAN, E., VAN'T LAAR, A., MOCHTAR, I. A. & VEDER, H. A. (1959). Deterioration of AHG (Factor VIII) and fibrinolysin. *Thrombosis et Diathesis Haemorrhagica*, **3**, 1–5.

BRINKHOUS, K. M., LANGDELL, R. D., PENICK, G. D., GRAHAM, J. B. & WAGNER, R. H. (1954). Newer approaches to the study of hemophilia and hemophilioid states. *J. Amer. med. Ass.* **154**, 481–486.

GRAHAM, J. B., MCLENDON, W. W. & BRINKHOUS, K. M. (1953). Mild hemophilia: an allelic form of the disease. *Amer. J. med. Sci.* **225**, 46–53.

HARTMAN, F. (1927). Changes in the clotting time of the blood of cats as a result of exercise. *Amer. J. Physiol.* **80**, 716–718.

MACFARLANE, R. G. & BIGGS, R. (1946). Observations on fibrinolysis. Spontaneous activity associated with surgical operation, trauma, etc. *Lancet*, **251**, 862–869.

MERSKEY, C. (1950). The consumption of prothrombin during coagulation. The defect in haemophilia and thrombocytopenic purpura. *J. clin. Path.* **3**, 130–141.

MILLS, C. A. & NECHELES, H. (1928). Variations in the coagulability of the blood normally and after food ingestion. *Chin. J. Physiol.* **2**, 19–23.

OWREN, P. A. (1947). The coagulation of blood. Investigations on a new clotting factor. *Acta med. scand.*, Suppl. 194.

PENICK, G. D. & BRINKHOUS, K. M. (1956). Relative stability of plasma antihemophilic factor (AHF) under different conditions of storage. *Amer. J. med. Sci.* **232**, 434–441.

POOL, J. G. & ROBINSON, J. (1959). Assay of plasma antihaemophilic globulin (AHG). *Brit. J. Haemat.* **5**, 17–23.

SCHNEIDER, R. A. & ZANGARI, V. M. (1951). Variations in clotting time, relative viscosity, and other physicochemical properties of the blood accompanying physical and emotional stress in the normotensive and hypertensive subject. *Psychosom. Med.* **13**, 289–303.

WAGNER, R. H., PATE, D. & BRINKHOUS, K. M. (1954). Further purification of antihemophilic factor (AHF) from dog plasma. *Fed. Proc.* **13**, 445–446.