Changes in volume and density of platelets in myocardial infarction

J F MARTIN, JULIE PLUMB, R S KILBEY, Y T KISHK

Abstract

The distributions of platelet volume and density were measured in 15 men suffering myocardial infarction and in 22 healthy controls. The method used separated 93% of the total platelet population from whole blood. Mean platelet volume of the study group compared with that of controls was increased by a mean of 0·98 fl ($p < 0·001$) in the first 12 hours after myocardial infarction, and by 1·24 fl six weeks later ($p < 0·001$). Distribution of platelet volume remained log normal after myocardial infarction. Modal platelet density was increased by a mean of 25 g/l ($p < 0·05$) after myocardial infarction. Platelet volume is probably chronically large in men suffering myocardial infarction and may be related to changes in megakaryocytes. It is suggested that the increase in platelet volume occurs before infarction.

Introduction

Platelets appear to play a part in both the production of atherosclerosis[1] and the formation of arterial thrombosis[2] but so far no consistent platelet abnormality has been found in vascular disease. Platelet volume has a wide range (2-40 fl) and can vary physiologically as a response to thrombocytopenia.[3 4] Platelet volume is determined at thrombopoiesis,[5 6] and increases in platelet volume are associated with increases in the DNA concentration in the nucleus of the megakaryocyte.[4 7 8] As discussed elsewhere, large platelets are not necessarily young platelets[9] and there is now no convincing evidence that platelets appreciably change volume or density as they circulate.[10 11] We investigated volume of platelets, and the related biophysical variable density, in patients who had suffered myocardial infarction.

Patients and methods

PATIENTS

A total of 15 men admitted to the coronary care unit were studied. Blood samples were taken from the cubital vein within 12 hours of the onset of chest pain. On admission all had classic electrocardiographic changes of myocardial infarction and the appropriate rise in serum creatine phosphokinase concentration and lactic dehydrogenase activity. The mean age was 55 years (range 38-65). Blood was also taken from a control group of 22 normal male doctors and laboratory workers at the same time. None of the controls complained of chest pain and all had normal electrocardiograms. The mean age was 44 years (range 25-65).

Six weeks later three of the 15 patients had died and one was lost to follow up; blood was taken as before from the remaining 11 and from the 22 controls.

University of Sheffield, Department of Medicine, Royal Hallamshire Hospital, Sheffield, S10 2JF

J F MARTIN, MD, MRCP, lecturer in medicine
JULIE PLUMB, BSC, research student
R S KILBEY, MB, CHB, medical registrar
Y T KISHK, MB, MS (MED), research fellow

Correspondence to: Dr J F Martin.

Platelet volume was measured on all blood specimens within three quarters of an hour of venesection during which time volume did not change.[5] A group of five normal male laboratory workers gave blood for daily measurement of distribution of platelet volume over five consecutive days and three of them again at four months.

ISOLATION OF PLATELETS

Nine volumes of blood were taken into one volume sodium citrate 38·0 g/l and prostaglandin E_1 0·4 mg/l.

Platelets were separated from whole blood using velocity centrifugation. The blood was diluted 1:1 in a solution containing KH_2PO_4 0·218 g/l, Na_2HPO_4 1·22 g/l, NaCl 6·832 g/l, $Na_3C_6H_5O_7.2H_2O$ 4·00 g/l, and glucose 2 g/l (BSGC); final pH 7·4; osmolarity 290 (1 SD) mmol (mosmol)/l, layered on to a 10 ml gradient of 50% (v/v) polyvinylpyrolidone coated silica (Percoll), self generated by centrifugation at 26 000 *g* for 20 min in a fixed angle rotor. The velocity gradient was centrifuged at 400 *g* for 10 minutes, leaving the platelets to be collected from near the top of the gradient. Percoll was adjusted to pH 6·5 and osmolarity 290 (1 SD) mmol (mosmol)/l for all gradients. Platelet counts were performed using a Coulter ZB counter. These correlated well ($r = 0·98$) with visual counts.

DENSITY OF PLATELETS

Distribution of platelet density was measured in 13 of the study group and 11 of the control group. Linear gradients of 20 ml of Percoll, spanning the density range 1030 to 1110 g/l were formed from a two chamber gradient maker. Platelet suspensions recovered from the velocity gradient described above were diluted 1:2 with BSGC and layered on to the linear gradients. Equilibrium centrifugation was at 2800 *g* for one hour. These conditions were chosen after pilot experiments had shown that after shorter times of centrifugation the platelet mass was still descending in the gradient whereas longer times did not produce an increase in observed modal density, indicating that equilibrium had been achieved at one hour. These conditions also approximate well to the theoretical equilibrium requirements.[10]

After centrifugation gradients were pumped from below and fractionated. Pumping speed was determined by pilot experiments that showed that the gradient could be reformed in a second tube without appreciable mixing if pumped at 1 ml/min. Platelet count and density were measured on each fraction of the gradients. Density was measured by dropping 5 μl aliquots of gradient material into a continuous linear kerosene-bromobenzene gradient, which had been calibrated using sucrose solutions of known density (calculated from the refractive index).

VOLUME OF PLATELETS

Aliquots of 10 μl of the above representative platelet population were placed in Isoton (Coulter Electronics) and platelet volumes were measured immediately. The apparatus for measurement consisted of a Coulter ZB particle counter coupled to an Apple II microcomputer by an analogue to digital converter, which excluded all signals longer than 20 μs and all signals with a bimodal form. Bimodal peaks are caused by more than one platelet being sized at one time and may lead to an artefactual distribution.[12] The computer was programmed to display a class histogram of platelet volume frequency in 256 channels and to perform statistical analysis of the distribution. The system was calibrated with latex particles of known volumes, (4·315 fl and 12·120 fl) and its linearity tested with a pulse generator. Even though the red blood contamination of the platelet population from the velocity gradient was minimal, the program was constructed to exclude all signals in the red cell volume range. This range was obtained by observing the volume distributions of a mixture of equal

numbers of platelets and red blood cells. Percoll particles, which have a diameter range of 15-30 nm, did not interfere with the volume measurement.

Results

Mean (SD) recovery of platelets from the velocity gradient was 93 (5%) (n=95) of the platelets in whole blood. Venous blood platelet counts were within normal range (150-400 $\times 10^9$/l) in the study and control groups. Figure 1 shows that mean platelet volume in five

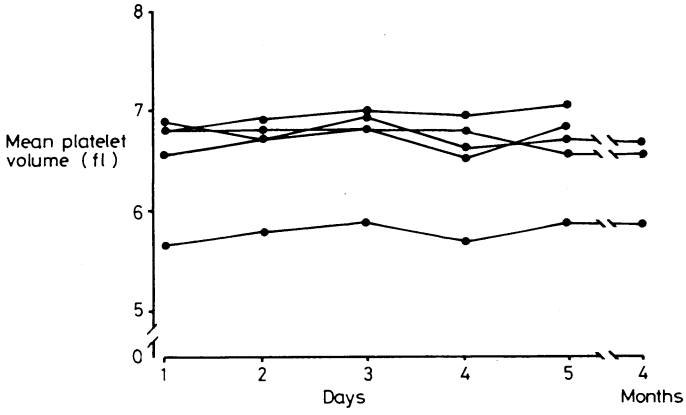

FIG 1—Mean platelet volume measured daily in five normal male subjects for five days, and in three again at four months.

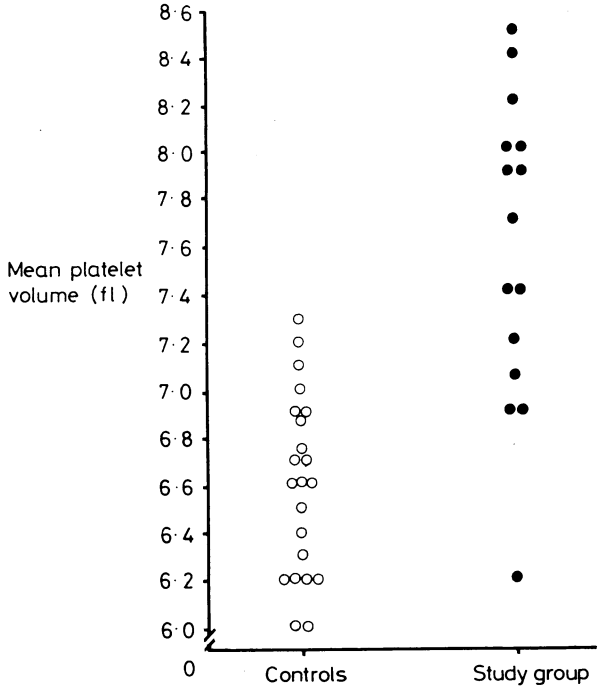

FIG 2—Mean platelet volume in 15 men suffering acute myocardial infarction and in 22 controls. In the study group platelet volume was measured within 12 hours of the onset of chest pain ($p<0·001$, Mann-Whitney U test).

normal men varied only slightly within each individual in five days and at four months mean platelet volume was unchanged in three individuals.

During the first 12 hours after myocardial infarction mean platelet volume was increased by a mean of 0·98 fl (14·9%) compared with that of the control group ($p<0·001$ Mann-Whitney U test) (fig 2). Six weeks after infarction the mean volume was still increased in the study group, this time by a mean of 1·24 fl (18·9%), ($p<0·001$) (fig 3).

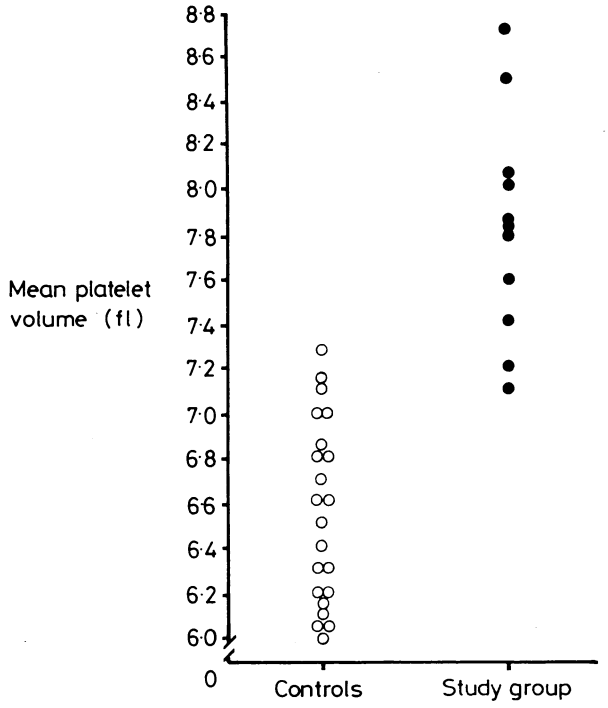

FIG 3—Mean platelet volume measured after six weeks in 11 of the study group and in the control group. Difference remains significant ($p<0·001$, Mann-Whitney U test).

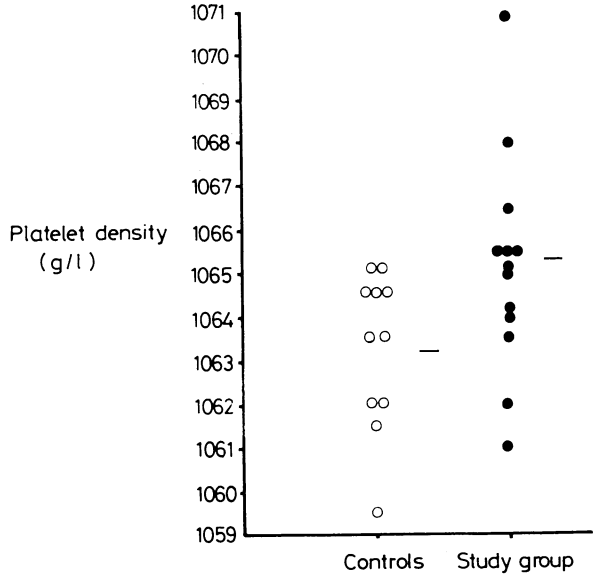

FIG 4—Modal platelet density in 13 of the study group (measured within 12 hours of the onset of chest pain) and in 11 controls. Density higher in study group ($p<0·05$, Mann-Whitney U test).

In the control group the mean modal platelet density was 1063 g/l; platelets from the patients within the first 12 hours after myocardial infarction had a mean modal platelet density of 1066 g/l, an increase of 6·25% of the platelet density range ($p<0·05$) (fig 4). Distribution of platelet density approximated to the normal in the control population as described elsewhere[13] and was similar in the study group. When the distribution of platelet volume was displayed as a log probability plot those of the study and control groups were both log normal (fig 5).

Discussion

Any study of platelets must give evidence that the whole platelet population is being tested. About 93% of the platelets

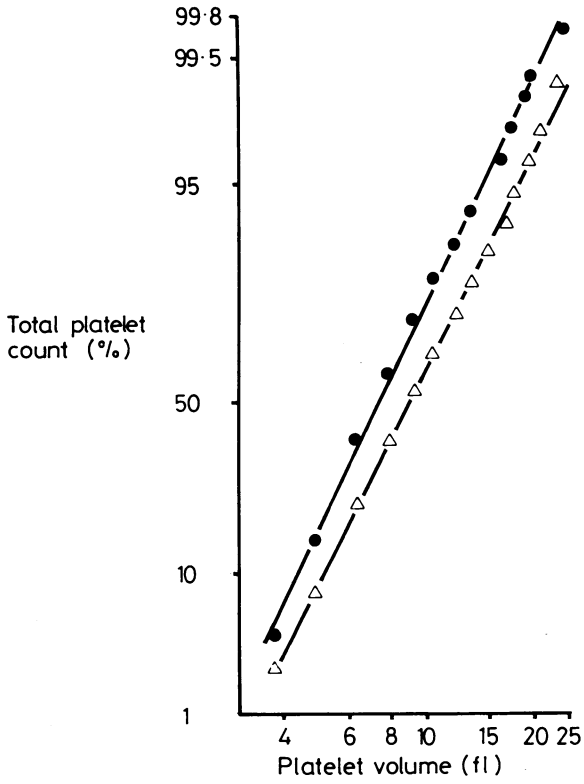

FIG 5—Log probability plot of mean platelet volume in patients with myocardial infarction (n=11) within 12 hours of onset of chest pain (▲) and in the control group (●). Log normality of distribution is maintained after myocardial infarction.

in whole blood were reaped by the isolation method used in this study. If platelet rich plasma were used to study platelet volume then some of the large platelets may well be lost from the test sample by the velocity gradient induced to prepare the platelet rich plasma from whole blood. Artefact may likewise arise in the study of distribution of cell volume. Those electrical pulses excluded will change the distribution curve. The electrical filtering in the system used here produces a distribution curve unaffected by the artefacts of cellular coincidence.[12]

Another source of possible error is that more active platelets in vascular disease may be activated and have altered shape, which would make them appear larger in a resistive particle sizing system. This has been a criticism of previous studies of platelet volume in vascular disease,[14] and has been shown to produce a decrease in skewedness of the platelet volume distribution curve.[14] Fig 5 shows that there is no decrease in skewedness using our conditions of anticoagulation, which were designed to inhibit platelet activation and not cause platelet swelling.[5]

Evidence from animal experiments and from reports on Eskimos in Greenland indicates that platelets may play a part in the production of atherosclerosis.[15-17] Others have suggested that a decrease in production of prostacyclin in endothelial cells is causally related to myocardial infarction,[18] and may entail changes in lipid metabolism. The production of the atheromatous coronary artery may be multifactorial but platelets almost certainly play a part, probably via secretion of a mitogen from alphagranules[19] or via other secretory products.

The final occlusive event leading to myocardial infarction may be a platelet thrombus; consistent abnormalities of platelet aggregation and biochemistry in myocardial infarction have, however, been difficult to document, and if they exist it has been argued that they are secondary to the infarction itself.

Platelet volume is a variable that is determined at thrombopoiesis.[5 6] The log normal distribution curve (fig 5) has been previously documented.[20] We found platelet volume to be increased in men suffering myocardial infarction when compared with controls. Although the mean age of the control group is lower than that of the study group, von Behrens has shown that human platelet volume does not increase with age over the age range studied here.[21] Thus the differences observed here should not be related to differences in the ages of subjects in the study and control groups.

Our findings suggest that mean platelet volume is increased before myocardial infarction occurs for three reasons. Firstly, the increase in volume seen within the first 12 hours of admission suggests that the increase was present before infarction, as the life span of the platelet is about eight days. More than 90% of the platelet population whose distribution was measured after myocardial infarction were circulating before the vascular occlusion occurred. Secondly, the fact that the increase persisted six weeks after discharge from hospital, when the infarct would have been largely healed, also supports the view that platelet volume was chronically larger in the infarct group. Finally, the preservation of log normality of platelet volume in the study group is added argument that the increase in volume was not due to consumption or production of a platelet subpopulation; the *whole* distribution curve was altered (fig 5). If a new larger population had been produced after myocardial infarction then it would appear as a deviation from log normality on the log probit plot. Differential consumption of small platelets would cause similar yet reciprocal change, as the log normal distribution of platelet volume is determined at thrombopoiesis.[5 6]

Our findings strongly suggest that the change in distribution of platelet volume arises at thrombopoiesis. The increase in platelet density seen within the first 12 hours of myocardial infarction is significant, yet slight compared with the increase in volume. The Gaussian distribution of platelet density is determined at thrombopoiesis.[6] Denser platelets have a higher mean volume than lighter platelets.[13] The increase in density seen here is that expected to follow an increase in mean platelet volume of the order described, whereas activation by the infarct would tend to decrease platelet density.

As platelet volume is determined by the megakaryocyte,[5 6] then platelet abnormalities in myocardial infarction may arise from megakaryocyte abnormalities. Megakaryocytes are polyploid cells; they have varying concentrations of DNA within the nucleus and they are capable of changing the relative distribution of DNA concentration.[7 8] Increase in platelet volume in animals has been shown to be associated with increase in the DNA concentration of megakaryocytes.[4] The evidence of change in platelet volume in myocardial infarction presented here indicates that myocardial infarction may be preceded by changes in megakaryocytes.

We thank Dr J Fleming for permission to study his patients; Dr E A Trowbridge for mathematical advice; Dr C Franks for designing and building the analogue to digital converter; Mr A Goude for programming the computer; Professor John Richmond for his support; and Mrs S Badger for preparing the manuscript.

References

1 Jørgensen KA, Dyerberg J. Platelets and atherosclerosis. *Dan Med Bull* 1980;**27**:253-9.
2 Mustard JF. Platelets and thrombosis in acute myocardial infarction. *Hosp Pract* 1972;**7**:115-28.
3 McDonald TP. The comparison of platelet size, platelet count and platelet ^{35}S incorporation as assays for thrombopoietin. *Br J Haematol* 1976;**34**:257-67.
4 Odell TT, Murphy JR, Jackson CW. Stimulation of megakaryocyte-opoiesis by acute thrombocytopenia in rats. *Blood* 1976;**48**:765-75.
5 Martin JF, Trowbridge EA, Salmon GL, Slater DN. The relationship between platelet and megakaryocyte volumes. *Thromb Res* 1982;**28**: 447-59.
6 Trowbridge EA, Martin JF, Slater DN. Evidence for a theory of physical fragmentation of megakaryocytes, implying that all platelets are produced in the pulmonary circulation. *Thromb Res* 1982;**28**:461-75.
7 Penington DG, Olsen TE. Megakaryocytes in states of altered platelet production: cell numbers, size and DNA content. *Br J Haematol* 1970; **18**:447-63.
8 Harker LA. Kinetics of thrombopoesis. *J Clin Invest* 1968;**47**:458-65.

[9] Martin JF, Slater DN, Trowbridge EA. Abnormal intrapulmonary platelet production: a possible cause of vascular and lung disease. *Lancet* 1983;i:793-6.
[10] Martin JF, Trowbridge EA. Theoretical requirements for the density separation of platelets, with comparison of continuous and discontinuous gradients. *Thromb Res* 1982;**27**:513-22.
[11] Martin JF, Penington DG. The relationship between the age and density of circulating 51-CR labelled platelets in the sub-human primate. *Thromb Res* 1983;**30**:157-64.
[12] Lloyd PJ, Scaplett B, Sinclair I. Effect of particle size and concentration on the response of a Coulter Counter. In: Groves MJ, Wyatt-Sargent JL, eds. *Particle size analysis*. London: Society for Analytical Chemistry, 1970:276-92.
[13] Martin JF, Shaw T, Heggie J, Penington DG. Measurement of the density of human platelets and its relationship to volume. *Br J Haematol* 1983;**54**:337-52.
[14] Laufer N, Grover NB, Ben-Sasson S, Freund H. Effects of adenosine diphosphate, colchicine and temperature on size of human platelets. *Thromb Haemost* 1979;**41**:491-7.
[15] Fuster V, Bowie EJW, Lewis JC, Fass DN, Owen CA, Brown AL. Resistance to arteriosclerosis in pigs with von Willebrand's disease. *J Clin Invest* 1978;**61**:722-30.
[16] Harker LA, Ross R, Slichter SJ, Scott CR. Homocysteine-induced arteriosclerosis. The role of endothelial cell injury and platelet response in its genesis. *J Clin Invest* 1976;**58**:731-41.
[17] Dyerberg J, Bang HO, Stoffersen E, Moncada S, Vane JR. Eicosapentanoic acid and prevention of thrombosis and atherosclerosis. *Lancet* 1978;ii:117-9.
[18] Dembinska-Kiec A, Gryglewska T, Zmuda A, Gryglewski RJ. Generation of prostacyclin by arteries and by the coronary vascular bed is reduced in experimental atherosclerosis in rabbits. *Prostaglandins* 1977;**14**:1025-35.
[19] Kaplan DR, Chao FC, Stiles CD, Antoniades HN, Scher CD. Platelet α-granules contain a growth factor for fibroblasts. *Blood* 1979;**53**:1043-52.
[20] Paulus JM. Platelet size in man. *Blood* 1975;**46**:321-36.
[21] von Behrens WE. Mediterranean macro-thrombocytopenia. *Blood* 1975;**46**:199-208.

(*Accepted 28 April 1983*)

Clinical experience with the oxygen concentrator

T W EVANS, J WATERHOUSE, P HOWARD

Abstract

The oxygen concentrator is an accepted means of delivery of long term domiciliary oxygen treatment. Conditions of use, however, need to be carefully defined. Fourteen concentrators were used for one year by patients with hypoxaemic chronic obstructive airways disease, and mechanical reliability, patient compliance with a regimen of 15 hours' use a day, smoking habits, and variation in arterial gas tensions studied.

Though many patients failed to achieve either the desired daily use or the recommended arterial oxygen tension, problems were generally minor and could probably be overcome by careful supervision and planning. Overall the concentrator appeared to be the most economical means of providing oxygen treatment at home and was much preferred by patients who had previously used oxygen cylinders.

Introduction

Patients with chronic obstructive airways disease, hypoxaemia, and oedema benefit from long term domiciliary oxygen treatment. Studies by the Medical Research Council and National Institutes of Health in such patients showed a substantial long term reduction in mortality.[1 2] To achieve this oxygen was required for at least 15 hours a day at flow rates sufficient to raise the arterial oxygen tension (Pao_2) to 8·0 kPa (60 mm Hg)—usually around 2 l/min. There are three methods of providing long term domiciliary oxygen: cylinders of 1360 l delivered to the patient's home (the most widely used method), liquid oxygen in a domestic tank replenished twice weekly (used only in Edinburgh in the United Kingdom), and the oxygen concentrator.

The oxygen concentrator is about the size of a small refrigerator and separates nitrogen from air on a "molecular sieve". It will deliver oxygen to the patient at concentrations of 90% and at flow rates up to 4 l/min.[3] The capital cost of the concentrator is £1200, which may be considered expensive in relation to the size of the patient population. Nevertheless, the yearly running cost of £150-200 is substantially less than the £4000 for providing cylinders and the £1500 for liquid oxygen.[4] Thus on cost alone the oxygen concentrator is the preferred means of providing long term domiciliary oxygen treatment. Although the machines are used world wide, they have not been tested under prescribed therapeutic conditions demanding 15 hours of use daily. This study was devised to assess the mechanical reliability of the concentrator, patient compliance, and the therapeutic efficacy of oxygen concentrators over a period of 12 months.

Method

Fourteen concentrators were allocated to patients with cor pulmonale secondary to chronic obstructive airways disease living in the Sheffield area. One machine was held in reserve at the hospital. Of the 15 concentrators, 12 were De Vilbiss $DeVO_2$ machines and three Rimer Birlec DOM 9 machines. At the beginning of treatment arterial gas analysis was performed to ensure that a Pao_2 of at least 8 kPa was maintained at a flow rate of around 2 l/min. Patients were instructed to use the machines for 15 hours a day and those who smoked cigarettes advised to stop in the strongest possible terms. Every three months technical staff visited the patients' homes to read the (hidden) clock, change air filters, perform minor servicing as appropriate, and ensure that the concentration of oxygen delivered by the machine was at least 90%. Servicing was carried out by a trained medical physics technician from our department in order that mechanical reliability could be more accurately measured than through the manufacturers' servicing arrangements. In the event of total failure of a machine, the patient was instructed to telephone our respiratory function laboratory. No back up oxygen cylinder was provided, as any machine requiring withdrawal from service was immediately replaced by the reserve concentrator.

Patients were seen at regular intervals in the outpatient department for clinical assessment. After the concentrators had been in use for 12 months we calculated the hours of use a day, allowances being

Department of Medicine, University of Sheffield, Royal Hallamshire Hospital, Sheffield S10 2JP

T W EVANS, BSC, MRCP, research fellow
J WATERHOUSE, respiratory technician
P HOWARD, DM, FRCP, reader

Correspondence to: Dr P Howard.