in the area was determined. It was found that 4,088 people over the age of 17 lived in the area, and that 38% (1,552) drove a motor vehicle. Of these drivers 76.6% (1,190) were patients of one group practice, and the known mental and physical illnesses of these drivers were determined from the practice records.

It was found that 9.2% (109) of the drivers had at least one significant mental or physical illness. The criterion of significance used was whether the illness rendered the motorist unsuitable to drive a public service vehicle. In the case of mental illnesses an additional requirement was that the illness had been present for at least three months.

Of the drivers 6.5% (77) had physical illnesses and 2.7% (32) had mental illnesses ; 7.4% (69) of the male drivers and 3.0% (8) of the female drivers had a significant physical illness ; and 2.2% (20) of the male drivers and 4.6% (12) of the female drivers had a significant mental illness.

Most of the drivers with physical illnesses were over the age of 50, the largest single grouping being in the sixth decade. The majority of those with mental illnesses were below the age of 50, with the largest single grouping in the fourth decade. Half the physically disabled drivers below the age of 40 had either epilepsy or blackouts, and with one exception all the epileptics were under the age of 40. The percentage of epileptics driving was similar to the proportion one would have expected to find driving if they did not suffer from their illness.

It was found that some severely disabled people continued to renew their driving licences, though they no longer continued to drive. It is probable that less than 1% of all motorists declare disability when applying for a driving licence.

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Diurnal Variations of Platelet Stickiness compared with Effects Produced by Adrenaline

EDWIN BESTERMAN,* M.A., M.D., M.R.C.P. ; GILLIAN MYAT,† B.SC. ; VIN TRAVADI‡

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The first simple method of assessing platelet stickiness was introduced by Wright (1941). Subsequent modification by McDonald and Edgill (1957) showed that this technique could be used to demonstrate a significant difference in platelet stickiness between patients with ischaemic heart disease and control subjects. The technique used in the present study is exactly that of McDonald and Edgill. Before beginning the present investigation reproducibility of results was adequately verified in a series of cases, both by the same investigator and in comparison with other investigators. In a comparison of patients with ischaemic heart disease and control subjects platelet stickiness results showed some scatter, but the mean difference between these two groups was significant at 0.01 level in our experience (Fig. 1).

Fat Loading

The present study was initially undertaken to investigate any correlation of changes of platelet stickiness with those of plasma lipids induced by a 50-g. fat breakfast.

In 1958 McDonald and Edgill studied patients with ischaemic heart disease before and after four to five weeks' subsistence on a fat-free fruit-and-rice diet ; reduced platelet stickiness occurred on this regimen. Mustard and Murphy (1962) found that a diet rich in dairy fat and eggs caused an increase of platelet clumping and diminished platelet survival times. However, there appear to be no reports on serial platelet stickiness changes in acute fat-loading studies.

Altogether 40 such fat loadings have now been carried out primarily in order to assess the effects of various drugs on

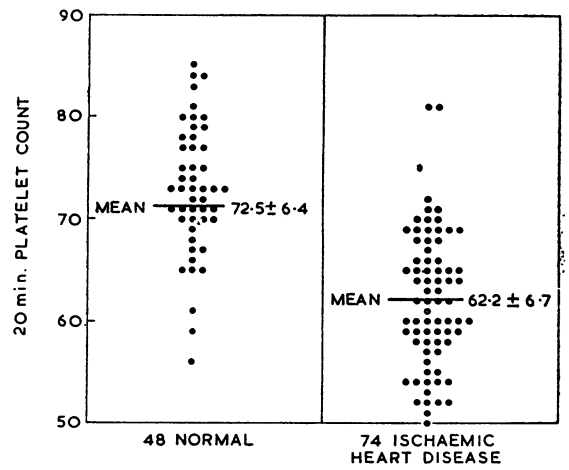

FIG. 1.—Morning platelet stickiness in 122 subjects.

* Consultant Cardiologist, St. Mary's Hospital, London W.2.
† Research Assistant, St. Mary's Hospital, London W.2.
‡ Technician, St. Mary's Hospital, London W.2.

hyperlipidaemia. After the first few triglyceride curves had been compared with the changes of platelet stickiness three unexpected findings were apparent: (*a*) though platelet stickiness was shown to increase in all cases, the maximum increase occurred later than the peak of induced triglyceridaemia; (*b*) the increased stickiness persisted after the elevated postprandial lipid levels had returned to normal; and (*c*) the degree of increase of platelet stickiness was unrelated to the level of hypertriglyceridaemia obtained, and occurred in control subjects as well as in those with ischaemic heart disease.

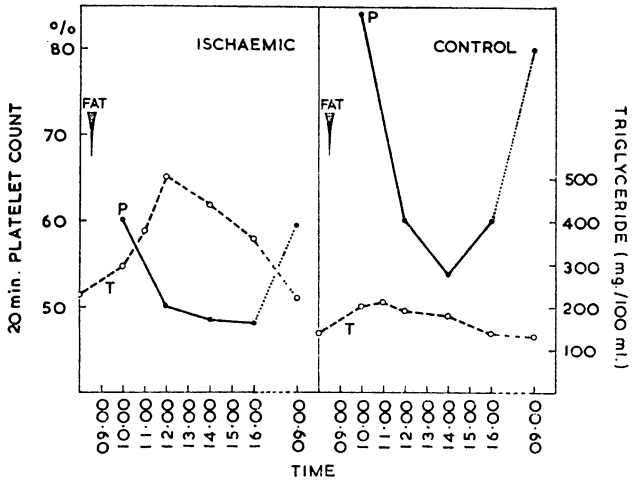

FIG. 2.—Triglyceride (T) and platelet stickiness (P) response to 50-g. fat breakfast.

In Fig. 2 two representative results of fat loading illustrate these points. The control subject showed little rise of triglyceride but an increase of platelet stickiness. A significant triglyceridaemia occurred in the ischaemic patient, but platelet stickiness continued to increase during the time that the triglyceride levels diminished.

In view of these findings platelet stickiness was then measured in the morning and afternoon without fat loading. As the stickiness was usually increased in the afternoon, a survey on a larger scale was undertaken.

Diurnal Variations

Platelet stickiness was measured between two and five times during one day on 89 occasions in 62 subjects (58 studies were carried out on 40 patients with ischaemic heart disease and 31 were made with 22 normal volunteers). A difference of 6% or more was regarded as a significant change of stickiness, and 12 subjects (eight patients and four controls) showed no such change. The remaining 80% showed a significant diurnal change of platelet stickiness, confirmed by repeating studies in many instances. Likewise, repeated measurements showed significant changes of stickiness, with and without fat loading. The mean results from all 89 observations are shown in Fig. 3. Similar values for stickiness were obtained between 09.00 and 11.00 hours; at 13.00 hours stickiness was increased to a significant degree ($P=0.05$) in both the control subjects and those with ischaemic heart disease. The increase was greatest at 15.00 hours, at which time the change of stickiness was highly significant ($P=0.001$). There were too few readings at 17.00 and 19.00 hours to include in a statistical analysis. At 17.00 hours eight out of nine ischaemic patients and two out of three control subjects still showed increased stickiness. By 19.00 hours the stickiness had returned to the morning values in the two patients and two normal volunteers studied at this time. Measurement at 09.00 hours the following morning confirmed the levels of stickiness found on the morning of the study.

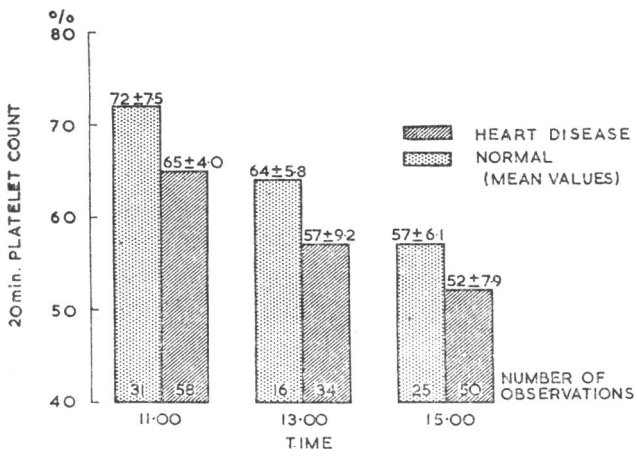

FIG. 3.—Diurnal variation in platelet stickiness in ischaemic heart disease and in normals (mean values).

Absolute values for platelet counts showed no significant differences in these diurnal studies.

Possible contributory factors to this diurnal change were then investigated. The effects of food were eliminated by 24-hour fasts failing to alter the diurnal change. Age, sex, menstruation, and physical activity had no apparent bearing on the diurnal rhythm. The only effect of disease was noted in those patients with acute cardiac ischaemia in whom the morning platelet stickiness was 50% or less: no diurnal changes were seen in these cases. Inpatient and outpatient diurnal swings were similar, but the absolute values for platelet stickiness were different. Twenty-seven comparative observations were made on 21 patients under these two conditions. The mean inpatient stickiness was 69% (±7.3) and the mean outpatient value was 63% (±4.7), significant at the 0.05 level.

In two unusually apprehensive subjects the morning platelet stickiness was greater than at 13.00 hours. In view of this observation and the extensive literature on catecholamine effects on blood clotting, this aspect appeared to justify further investigation.

Adrenaline Studies

Using adrenaline in animals, Wright (1944) showed increased platelet counts, and Nordøy and Rørvik (1965) found increased platelet adhesiveness. In vitro Waldron (1951) demonstrated accelerated clotting with both adrenaline and noradrenaline. Clayton and Cross (1963) showed a similar effect on platelet aggregation, using Born's (1962) technique. They also showed that this effect was similar to the aggregation produced by equimolar concentrations of adenosine diphosphate and was unaffected by heparin but partially prevented by alpha- and beta-blocking compounds. Mitchell and Sharp (1964) found that both amines accelerated platelet clumping. Rowsell *et al.* (1966) used extracorporeal shunts to show the effects of adrenaline on thrombus formation. O'Brien (1963) showed that isoprenaline did not have the same effects as adrenaline and noradrenaline on platelet aggregation in vitro. He also suggested (O'Brien, 1964a) that these catecholamine effects might be due to the stimulation of an adenosine triphosphatase.

As we could find no reports of the effects of small doses of adrenaline in vivo on platelet stickiness, a small trial was undertaken. Most animal and in vitro studies have employed fairly large doses of adrenaline; a near-physiological dose of 25 μg. was used in our study. This amount was injected subcutaneously in 12 volunteers, and in 10 a pronounced increase of platelet stickiness occurred between half an hour and two hours later (Fig. 4). Absolute values for platelet counts were unaltered, the mean levels being 213,000 before and 214,000 after

adrenaline. One splenectomized subject responded to adrenaline with a similar increase of stickiness.

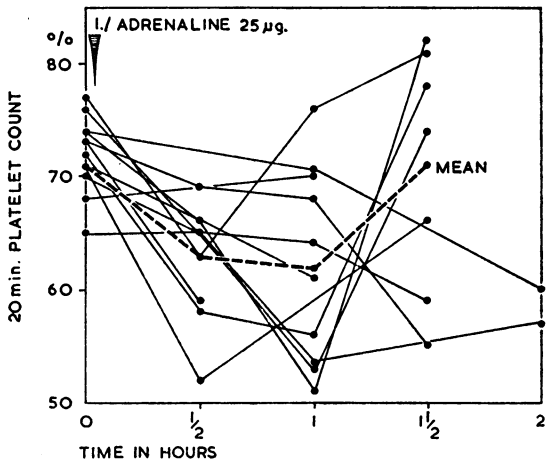

FIG. 4.—Adrenaline-induced changes in platelet stickiness.

An analysis was made of the mean levels of stickiness before injection and at the maximal level of change in all 12 subjects. Respectively these were 72%(±3.3) and 57%(±5.5); P=0.001.

The similarity of these changes with those found in diurnal studies of control subjects is shown in Fig. 5. In view of these similarities, a possible diurnal catecholamine rhythm was regarded as a factor that might be responsible for the diurnal change of platelet stickiness.

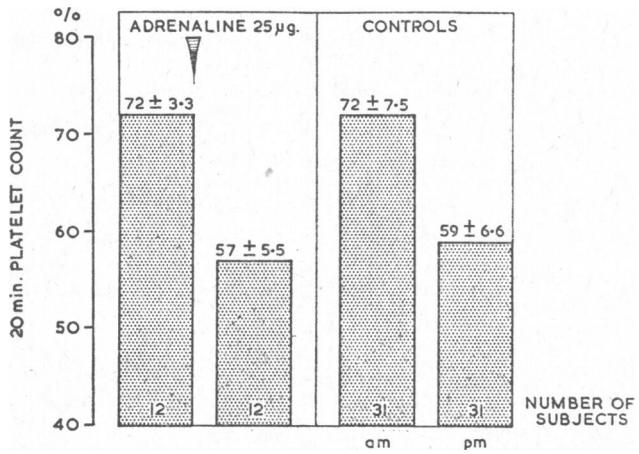

FIG. 5.—Adrenaline effect on platelet stickiness compared with diurnal change—in normals (mean values).

Catecholamines

Increase in urinary catecholamines during stress was first shown by Euler and Lundberg (1954). Since then many studies have been published on the effects of physical, intellectual, and emotional stress on catecholamine excretion. The results have been somewhat conflicting in regard to the relative increases of adrenaline and of noradrenaline in these situations, and variability between subjects is often considerable. Levi (1967) has shown that there is a quantitative relation between the strain experienced by an individual and his urinary excretion of adrenaline. Euler (1964) believed that adrenaline excretion was increased by stress, whereas noradrenaline increased with physical work; even the change from recumbency to the upright posture induced a threefold rise of noradrenaline output. Everyday work stress also produced increased noradrenaline excretion (Levi, 1967). Friedman *et al.* (1960) showed increased daytime noradrenaline excretion in a particular personality type, but even the control group showed a greater daytime output than that found at night. Elmadjian *et al.* (1957) also reported a diurnal variation of noradrenaline excretion in normal subjects. Under highly abnormal stress conditions Levi (1966) showed a diurnal rhythm of adrenaline excretion with the peak at midday and trough at midnight, even when the subjects did not sleep for 72 hours.

Catecholamine levels were therefore assayed in three three-hourly collections of urines from 10 subjects. These estimations were carried out by L. E. Martin, using a chromatographic separation similar to that described by Bertler *et al.* (1958), followed by fluorimetric assay. No free adrenaline was detected in any of the samples, but two that were hydrolysed showed significant quantities of conjugated adrenaline (6.75 µg. and 24.45 µg. respectively in the samples containing the least and greatest quantities of free noradrenaline).

Free noradrenaline was found in all samples, and in seven of the 10 subjects excretion of this amine was increased in either the second or third sample. Collections were made from 07.00 to 10.00, 10.00 to 13.00, and 13.00 to 16.00 hours. The mean levels at each collection are shown in Fig. 6. The difference between the mean levels of noradrenaline in the first and second samples is barely significant (0.05 to 0.1), but the difference between the first and third samples (19.2 and 25.4 µg.) is significant at the 0.001 to 0.01 level.

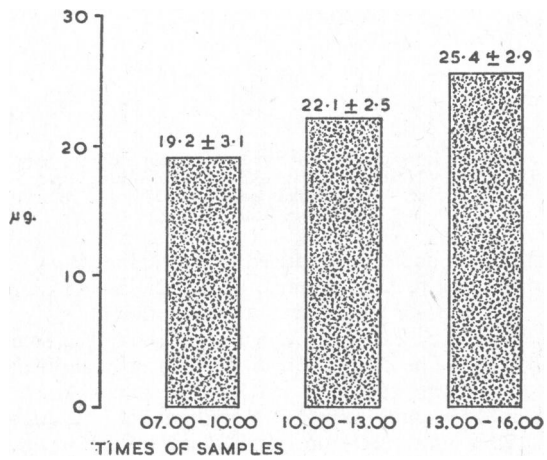

FIG. 6.—Mean urine noradrenaline concentrations in 10 subjects (three three-hourly collections).

Biological assay for catecholamines has also been carried out on three samples from each of three subjects. These results were entirely different from those found on chemical assay. Free adrenaline was present in all samples; the quantities of free noradrenaline were less than a third of those detected chemically. No diurnal changes were present.

Discussion

The differences in serial studies of platelet stickiness found in any one subject are considerable. Fig. 7 shows the changes found during a period of 15 months in two normal subjects and in two patients with ischaemic heart disease. Nevertheless, a mean difference exists between the stickiness in ischaemic subjects as compared with controls.

Philp and Wright (1965) found increased platelet stickiness two hours after a fatty meal, associated with a significant decrease of platelet count. We did not observe this change in absolute numbers. As the populations receiving fat loads and those investigated without fat loads were not strictly comparable, our results cannot exclude an added effect from lipaemia. The continued effect on postprandial platelet stickiness after the peak of lipaemia had passed is similar to the delayed red cell aggregation and platelet clumping found in hamsters after a fatty meal (Cullen and Swank, 1954).

The unexpected finding of a diurnal swing in stickiness irrespective of fat loading accentuates the need for blood sampling at the same time of day in comparative studies, preferably before 11.00 hours. In a study of this diurnal change nine subjects also had their platelet aggregation measured by the Hellem (1960) technique ; no comparable change was found. However, the Hellem method did show increased aggregation of platelets after injection of adrenaline. This discrepancy remains unexplained. The effects of small doses of adrenaline in man are not due to the release of immature " sticky " platelets as has been suggested by certain animal studies with higher doses of adrenaline. The present study confirms in-vitro results, suggesting that catecholamines affect platelet aggregation. In view of the similarity between the diurnal change in

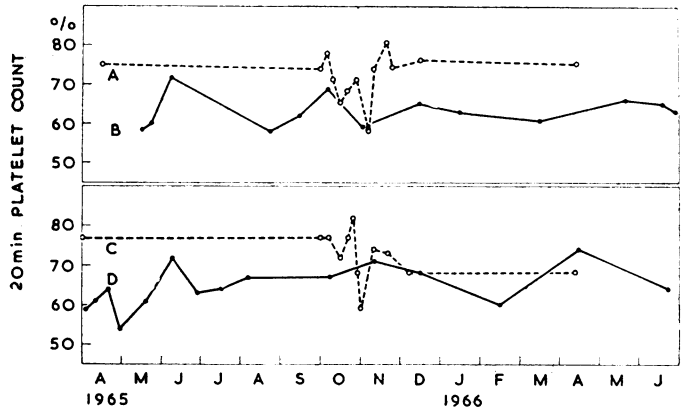

FIG. 7.—Serial platelet stickiness observations in four subjects (April 1965 to June 1966). A=Normal woman aged 26. B—Man aged 54 with ischaemic heart disease. C=Normal man aged 30. D—Man aged 56 with ischaemic heart disease.

platelet stickiness and that produced by adrenaline, it was hoped to demonstrate a parallel increase of catecholamine excretion. However, no free adrenaline was detected, though early afternoon noradrenaline excretion was higher than that found in early morning. The proportion of subjects showing a diurnal change of platelet stickiness was 80% ; adrenaline effects occurred in 84% and increased noradrenaline excretion was shown in 70% of subjects on chemical assay. However, only small numbers were involved in the latter two investigations.

In serial studies on platelet aggregation after adrenaline in vitro O'Brien (1964b) found variable responses in each subject over a period of time. He suggested that this might be caused by a fluctuating population of adrenaline-resistant platelets. Subsequent studies (O'Brien, 1965) suggested a different mechanism of in-vitro aggregation produced by adenosine diphosphate as opposed to that caused by added adrenaline.

Diurnal swings of adrenaline excretion have been demonstrated by Levi (1966) and daytime increases of noradrenaline have been shown by others (Friedman *et al.*, 1960). The normal stresses of everyday work studied by Levi (1967) were reflected by greater changes of noradrenaline than of adrenaline secretion. These findings are more apposite to the conditions under which our subjects were studied.

The conflicting results of the chemical and biological assays make it impossible to draw any conclusions concerning diurnal changes in catecholamine excretion from the present study. However, the results of other catecholamine studies suggest that such changes exist. Thus a possible relation between diurnal catecholamine output and changes in platelet stickiness remains an unproved hypothesis.

Summary

A significant increase of platelet stickiness has been shown to develop in the afternoon in 80% of both normal subjects and patients with ischaemic heart disease.

Adrenaline, 25 μg. subcutaneously, has induced a similar but transitory change. It is suggested, but not proved, that this diurnal change of platelet stickiness is associated with a diurnal rhythm of catecholamine release.

We are much indebted to L. E. Martin and J. J. Brown for carrying out the catecholamine assays.

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