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Reduction in blood platelet size with increase in circulating numbers in the postoperative period and a comparison of the glass bead and rotating bulb methods for detecting changes in function

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SYNOPSIS The changes in blood platelet numbers, size, adhesiveness, and response to adenosine diphosphate have been followed for about a month after major operations performed on 19 patients.

The well established increase in numbers was confirmed and reached a maximum of rather less than double the normal numbers in the third week. It was accompanied by a reduction of platelet size from $8{\cdot}3\mu^3$ to $7{\cdot}2\mu^3$, and by an increased responsiveness shown in all the tests employed. These abnormalities were preceded by an initial change in the reverse direction from that obtaining at the peak of the response.

In this series the bead method proved superior, as a means of delineating the postoperative platelet response, to the rotating bulb method in tests of timing, proportional changes, and of discrimination.

A previous series of tests has shown that the platelets of patients with ischaemic heart disease were larger than those of the control subjects (Gooding and Enticknap, 1967a and b, and Enticknap, Gooding, Lansley, and Avis, 1969). Serial estimations following acute necrosis of the myocardium showed that, with recovery, these large platelets became progressively more normal in size. This abnormality may be either a primary effect of the disease or secondary to the excessive deposition of thrombi causing the surviving platelets to be either younger or older than those in the controls. To elucidate this we have determined cell sizes in the postoperative period. At this time release of platelets into the circulation is known, predictably, to increase and it is reasonable to assume that this reduces the average age of the circulating cells. The results presented here show that these young cells are smaller. The opportunity was also taken to delineate other aspects of the postoperative response in order that those appropriate to testing of thrombolytic drugs could be recognized and to determine whether the glass bead method of Hellem (1960) or the rotating bulb method modified from Payling-Wright (1941) was the more effective in detecting the response.

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Methods

The methods have been fully described (Enticknap *et al*, 1969) and only minor modifications have been required in the four years that they have been in use. Platelets are now counted in the range 16-100 divisions (Coulter counter, model B) but as there appears to have been a minor shift in the calibration this still corresponds to 3-17μ^3. The conversion factor determined each day instead of the mean figure over a period is now entered separately into each computer run. The Hellem tubes were from a single batch made at one time and so have aged somewhat in the year of these experiments. The term 'platelet size' refers to geometric mean volumes calculated in an IBM 1130 on a specially written programme.

Parameter Measured	*Preoperative Period*	*Postoperative Period*							
		Days 1-3	*Days 4-6*	*Days 7-9*	*Days 10-12*	*Days 13-14*	*Third Week*	*Fourth Week*	*Fifth Week and After*
No. of tests	24	11	15	13	18	7	10	5	3
Total platelet count $\times 10^{-3}$	319·9±82·3	308·5±68·0	287·8±93·1	358·6±114·9	409±146·3	443·7±203·7	498·1±178·1	367·4±104·4	383·8±174·1
Total platelet size in μ^3	7·735±0·74	8·272±0·53	8·141±0·63	7·938±0·75	7·636±0·51	7·314±0·55	7·217±0·67	7·440±0·92	8·247±0·40
Adhesive platelet count $\times 10^{-3}$ bulb method	252·7	243·3	225·0	270·9	272·6	374·3	362·3	274·9	229·8
Adhesive platelet size in μ^3 bulb method	7·735	8·262	8·005	8·155	7·783	7·293	7·25	7·29	8·23
Adhesive platelet count $\times 10^{-3}$ bead method	177	168	197	206	195	229	275	169	251
Adhesive platelet size in μ^3 bead method	7·725	8·42	8·14	8·02	8·00	7·24	7·19	7·46	8·27
Ratio of adhesive counts by bulb and bead methods	1·68	1·46	1·20	1·35	1·42	1·65	1·52	1·43	1·17
ADP aggregation Percentage aggregated in 15′	70·47	61·01	70·83	74·30	78·61	64·05	70·39	57·06	38·03
Opacity change $\frac{1\text{cm}}{600} \times 10^3$	27	20	24	25	27	28	30	21	17
Opacity fall in 1′ dE $\frac{1\text{cm}}{600} \times 10^3$	12	7	8	9	13	14	13	8	6
ADP-lag (Secs)	18±9·8	37±46·1	23±7·9	18±21·7	20±14·0	19±16·5	19±11·0	13±3·9	13±17·0

Table *Mean values and some standard deviations in measurements of platelet parameters*

Subjects

The platelet responses to 19 major operations were studied. Two were splenectomies, one patient was treated with heroic doses of dipyridamole, and another had a platelet abnormality comparable to idiopathic thrombocytopenia. The remaining 10 men and five women were drawn from a wide unselected group. One to four preoperative tests were done and four to 10 postoperative tests, at clinically convenient times, to give a total of 106 tests. The average age of the subjects was 61·6 years ±9·3.

Results

The major findings are set out in the Table which shows the inverse relationship between numbers and size of platelets. There is remarkably close similarity between the changes in the counts of adhesive cells and of the total counts, and of the size measurements on both adhesive cells and their parent populations. The total response to adenosine diphosphate (ADP), the initial rate of response, and the percentage of cells responding all follow the pattern of the total cell count, but the delay (ADP lag time) in responding, after doubling in the first few days after operation, falls slowly again to normal. The reduction in mean platelet size is clearly progressive throughout the earlier part of the period. To test the significance of the change the value of the mean of the observations made in the first three days has been compared with the mean in the third week and the reduction is highly significant

$$(t = 3{\cdot}873 \text{ for 19 d.o.f.}; >0\ 001\ \text{P}<0{\cdot}01).$$

Comparison of the two methods of measuring adhesiveness, referred to below as the bead (after Hellem) and bulb (after Payling-Wright) methods, shows significant differences. The bulb method when used in this series gave higher adhesive counts by an average ratio of 1·36 but this depended upon the rate of rotation or of pumping. The level can be set between about 10 and 90% adhesiveness at will by varying the rates. In the first few postoperative days a change, which is in a reverse direction to the major change, has been found in all parameters. This change itself reaches a peak in the third week. In the bulb method the reversal occurs in the second three-day period but the bead method shows it already in the first three days. This appears as a fall from 1·66 to 1·20 in the ratio between the two counts, which climbs again to 1·65 in the second week, indicating that the two tests delineate the change with equal effectiveness at the peak period although the bead method does so a little earlier. Another way of testing the efficacy of the two tests is to determine which gives an increase of, say, 30% over the preoperative level at an earlier stage. The bead method did so in seven cases at an average of 10 days, the bulb in two only though at an average of four days. In the rest of the cases the rise was on the same day. Similarly, the result from the bulb method increased by 30% earlier than did the total count on only two occasions and was later in five subjects, whereas the postbead adhesive count reached 1·3 × the control value earlier in seven subjects and later only in one. Thus the bead method starts to respond earlier, reaches a reasonably certain increase earlier, and more often gives more information than the total count. But, can it always delineate the peak so clearly? Disregarding the cases in which no peak was seen in the period of study, in six of the other cases the simple total count gave a peak on the same test day as both methods, in six the bead method reached a peak earlier by an average of seven days, and in two of these the bulb method only gave its peak very late. Thus the bead method gives its peak earlier and more certainly. The mean peak times were for the total count 12·1 ± 2·2 days, for the bead method 10·8 ± 3·8 days, and for the bulb method 14·3 ± 4·2 days with average ratios of maximum count to preoperative average count of 1·68, 1·72, and 1·78 respectively. Thus the only advantage of the bulb method is a higher proportional increase at the peak, but this is no more easily detectable by simple discriminatory tests. The degree of correlation between the percentage adhesiveness measured by bulb and bead methods was, however, significant

$$(r = 0{\cdot}301 \pm 0{\cdot}97 : > 0{\cdot}001 \; P < 0{\cdot}01).$$

Two young boys have been studied following splenectomy. They have not been included in the overall averages but the results of their tests were very similar to the general pattern. The cells decreased in size in one of them from $9{\cdot}44\mu^3$ to $9{\cdot}21\mu^3$ while the count rose to 568,000 per cmm and in the other from $9{\cdot}66\mu^3$ to $8{\cdot}27\mu^3$ during a change from 168,000 to 673,000 per cmm.

The difference in size between adhesive and non-adhesive cells was not marked in this series and the average values for 108 measurements of $7{\cdot}75\mu^3$ for all cells and $7{\cdot}89\mu^3$ for adhesive cells were not significantly different. However, Table I shows that these averages conceal a changing relationship in which the adhesive cells tend to be larger at first and smaller at the time of minimum overall size. Thus the preoperative values are very similar at $7{\cdot}81\mu^3$ for adhesive cells and $7{\cdot}74\mu^3$ for the whole population while those at the end of the second week are $7{\cdot}24\mu^3$ and $7{\cdot}42\mu^3$ respectively.

Discussion

It has long been known (Hueck, 1926) that the blood platelet count increases in the postoperative period and some other aspects of the response have been recently reinvestigated. Emmons and Mitchell (1965) showed increased initial rate and maximum intensity of the ADP response. Ham and Slack (1967) demonstrated a fall in platelet count preceding the rise which was, however, accompanied by increased adhesiveness and suggested that changes in function were established by the second day. Their series was carefully selected to exclude complicated cases. Hampton and Mitchell (1966) showed that there was associated change in electrophoretic behaviour of platelets which was at a maximum on the first postoperative day. Recently Bennett (1968) showed an increase in both total count and adhesiveness as early as four hours after operation and suggested that the latter is mediated by red cell changes. This investigation is, however, the first of which we are aware in which the cell size has been described and has demonstrated unequivocally a highly significant reduction at the peak of the response. This can leave little doubt that young platelets are smaller than older ones and that senescence, unlike in many other cytopoietic lines, is associated with increasing size. In addition the initial change in so many parameters in a reverse direction to that seen at the peak of the response has not been previously commented upon. Although it runs directly counter to the findings of Bennett (1968) it derives some support from the observations of Ham and Slack (1967).

The present comparison of the bulb and bead methods shows that the latter is the more suitable for detecting a postoperative response. In tests of timing, proportional changes, and discrimination it proves superior to the bulb method. It is only fair to comment that our usage does not follow exactly that of the originator, but the major distinction between adhesion to a large mobile surface over many minutes and to closely packed stationary beads for a few seconds, with the attendant turbulence phenomena, in our view, leaves the comparison valid. It accords too with our previous finding that the bulb method gave no better discrimination between subjects with ischaemic heart disease and controls (Enticknap *et al*, 1969).

A possible explanation of our major finding, reduction in cell size with increasing peripheral blood platelet count in the postoperative period, may indeed lie in an inverse correlation between these two parameters, either of biological or methodological origin. In our previous large series many such correlations were examined, both between plasma counts and blood counts and the six parameters of platelet size that were then studied. As there existed in those series a reasonable degree of correlation in the subjects with ischaemic heart disease and in the controls, in whom there were no major changes in the blood counts, and no clear-cut differences between the closeness of the correlations in diseased and control subjects, this appears to be a fundamental relationship. However, in postoperative subjects, many of whom had some degree of arteriosclerosis by virtue of age, the degree of inverse change is much greater than could be explained by the loose correlation previously seen. This cannot therefore be the whole of the explanation and the decrease in cell size must be real and related to a changed platelet economy.

In the same investigation we demonstrated that adhesive cells were significantly smaller than non-adhesive cells. In control subjects the difference was $0{\cdot}54\mu^3$. In this series we have not calculated the size of non-adhesive platelets so the only comparative figure available is the smaller difference between total and adhesive populations of $-0{\cdot}14\mu^3$, which in the previous series was $+0{\cdot}16\mu^3$. However, while the preoperative difference was minute ($-0{\cdot}07\mu^3$), by the end of the second week, when a large number of new small cells had been produced with a higher proportion than normal of adhesive cells, there was a normal difference of $+0{\cdot}18\mu^3$. Thus the most likely explanation of the data is that both adhesive cells and new cells are in fact smaller than the average size, though the differences between them are slight; and further, that the two changes tend to be associated, the adhesive cells being the younger ones. This conclusion, based on size data, accords with that to be drawn from the higher proportion of the increased postoperative platelet count which is in the earlier stages (of newer cells) made up of more adhesive cells. The subsequent increase in cell size is yet further support for this interpretation. Hirsh, McBride, and Wright (1966) concluded that the bulb and bead methods measured substantially the same aspect of platelet function. However, their data contained various forms of both animal and human material and the propriety of calculating a single correlation based on such diverse data is arguable. The correlation they showed was largely due to the very low values derived from manipulated rabbit plasma; the closely related normal figures in man would not have revealed it. In our own previous comparison we found a correlation coefficient of $r = 0{\cdot}17$ between percentage adhesiveness measured by the two methods, which was only just significant at $p < 0{\cdot}05$. In this investigation the correlation was better but again low, although now spread over a much wider range of activity. Though it may well be that the methods do measure the same aspect of platelet function (presumably the ability of glass surfaces to activate an adenosine triphosphate system) this investigation has clearly demonstrated the greater efficiency of the bead method in the postoperative situation.

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