Heparin neutralizing activity test in the diagnosis of acute myocardial infarction

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SYNOPSIS We have previously shown in patients after recovery from a myocardial infarct (post-MI) that the heparin neutralizing activity (HNA) in the plasma is raised and that this activity may come from platelet factor 4 derived from activated platelets. This report concerns 89 patients admitted with acute chest pain; in 54, with evidence of acute infarction, the level of HNA is much higher than in a post-MI group or controls. Over the ensuing weeks the HNA decreases to the post-MI level. In 34 patients the evidence subsequently collected excluded a diagnosis of infarction; in these there were almost always normal amounts of HNA and little overlap with the results from the patients with infarcts. This easy test is therefore likely to prove clinically useful. Its significance is discussed.

The platelet count and platelet volume are both abnormal in patients with acute infarction and also in the chest pain group so these tests do not help to discriminate.

There are several reports, including those recently summarized (O'Brien, 1974), that in patients with acute myocardial infarction (ac-MI), in patients after recovery from infarction (post-MI), and in patients with atherosclerosis there is an increase in various tests probably measuring heparin neutralizing activity, and we have now shown repeatedly that our test shows increased HNA in post-MI patients and in those with intermittent claudication (O'Brien, 1974; O'Brien et al, 1974a; O'Brien et al, 1974b). Furthermore, we have produced evidence that in at least some situations this HNA test measures platelet factor 4 (PF4) derived from activated platelets (O'Brien et al, 1974b). The present study concerns acute disease, namely 89 patients admitted with acute chest pain. The majority had acute myocardial infarction but inevitably in some the evidence collected later excluded this diagnosis. Patients with an acute attack of angina constitute an important and difficult diagnostic problem. Have they or have they not got a true infarct? Any additional diagnostic test would be useful.

Subjects

The intensive care unit reported to us 89 patients admitted with suspected ac-MI. The diagnosis given on the discharge letter was accepted only after reassessment of all the evidence by us. In one patient a firm diagnosis was impossible and he has been excluded. In 54 patients a definite diagnosis of infarction was made and these patients were kept in hospital for at least 10 days. In 34 patients the evidence excluded infarction and a diagnosis of acute angina was usually made; these patients were discharged after sufficient observation to ensure a correct diagnosis, usually in seven days. For all patients studied there was usually available a detailed clinical history, enzyme assays lactic dehydrogenase and serum aspartate aminotransferase performed at least twice, electrocardiogram and white counts. The control volunteers and post-MI patients were studied concurrently and some of these have been previously reported (O'Brien et al, 1974b).

The Tests

A test for heparin neutralizing activity (HNA) has been described previously (O'Brien et al, 1974b). If heparin is added to platelet poor plasma and none is neutralized then, when thrombin is subsequently added, a long clotting time will result. If a shorter time is observed then increased HNA is said to be present. Blood diluted 1 in 10 with trisodium citrate 3.8% is centrifuged within 10 minutes of collection...
in a BTL swing out centrifuge at 3500 rev/min (g = 1900) for 10 minutes at room temperature. The supernatant two-thirds of the platelet poor plasma (PPP) is removed, thus ensuring that the platelet layer is not disturbed. The platelet count of this PPP varies from 0 to 10 000/μl. The PPP can be stored frozen (−18°C) but was normally used within 2 hours. In this preparation only plastic or siliconed glass-ware were used. Clotting was performed in glass tubes 10 x 75 mm. To 0·1 ml of PPP warmed to 37°C was added 0·1 ml of heparin (Evans Pularin) 0·8 U/ml and the mixture was shaken. Twenty seconds later 0·1 ml thrombin 10 U/ml (Lister Institute) was added. The clotting time was always carried out in duplicate and the mean value was taken. The end point is initially difficult to see, but with practice this becomes easy.

Because the patient's own plasma acts as substrate in this HNA test, a normal thrombin time is an essential prerequisite, and in this series the thrombin clotting time was always normal (mean for the ac-MI group was 23·7 seconds; mean for the angina group was 23·7 seconds). To 0·1 ml PPP was added 0·1 ml thrombin 1·0 U/ml, and the clotting times of duplicates were recorded. The reproducibility of this method from month to month was ensured as follows. A control pool of PPP was prepared from apparently healthy donors and frozen in 0·5 ml aliquots at −18°C. Daily when the test was performed both a thrombin clotting time and the HNA test were carried out, and the results had to agree within 2 seconds from day to day. The heparin powder was weighed out to prepare a solution of 40 U/ml according to the manufacturer's declared strength. This stock was kept at 4°C and was stable for 6 to 12 months. Daily this stock is diluted to 0·8 U/ml using normal saline. Lister Institute human thrombin powder is dissolved in normal saline and diluted to produce a thrombin clotting time with normal plasma of 23 seconds. This is approximately 1 U/ml. For the heparin thrombin clotting time the thrombin is used 10 times stronger. Within one batch the reproducibility is excellent, and different batches are carefully overlapped. Bovine thrombin (Diagen) has been shown to give identical results. One hundred and forty apparently healthy 'normal' controls had a mean heparin thrombin clotting time of 29·6 ± 7·2 (2 SDs) and the mean thrombin clotting time was 23·2 ± 2·8 (2 SDs). It is probably better to aim at producing clotting times of this order rather than to accept slavishly the maker's declared strength of heparin or thrombin.

The whole blood platelet count (Coulter Model B) and the mean platelet volume (MCV Computer) were also measured relative to latex beads 2-02 μm diameter supplied by the makers. All patients were studied as soon as possible after admission (mean time after the episode 48 hours) and often several times in the next 14 days; 12 were followed for up to three months.

Results

HEPARIN NEUTRALIZING ACTIVITY

Acute myocardial infarction

Fifty-four patients subsequently accepted as having ac-MI gave HNA results of 8 to 28 seconds with a mean of 12·8 seconds when first tested, between the first and the seventh day after the episode (fig 1 and table). These times may be compared with control

![Graph](https://example.com/graph.png)

Fig 1 Scattergram of the heparin neutralizing activity clotting times in seconds (a short time indicates increased activity) in the three groups of patients and controls. The mean and two standard deviations is indicated. [Reproduced from the Lancet (1975), ii, 457 by courtesy of the editor]
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<table>
<thead>
<tr>
<th>Units</th>
<th>Acute MI</th>
<th>Chest Pain</th>
<th>Post-MI</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-7 days</td>
<td>8-28 days</td>
<td>1-3 months</td>
<td></td>
</tr>
<tr>
<td>HNA clotting time</td>
<td>sec</td>
<td>12-8 ± 6-98</td>
<td>14-2 ± 4-78</td>
<td>19-8 ± 6-19</td>
</tr>
<tr>
<td>Platelet count</td>
<td>× 10^8/l</td>
<td>189-00 ± 92-00</td>
<td>272-00 ± 127-00</td>
<td>195-00 ± 58-00</td>
</tr>
<tr>
<td>Mean platelet volume</td>
<td>fl</td>
<td>9-64 ± 3-04</td>
<td>9-08 ± 2-75</td>
<td>10-02 ± 2-42</td>
</tr>
</tbody>
</table>

Comparisons: *P Values

<table>
<thead>
<tr>
<th>Units</th>
<th>Controls v Acute MI (1-7 days)</th>
<th>Controls v Chest Pain</th>
<th>Controls v Post-MI</th>
<th>Acute MI v Chest Pain</th>
<th>Acute MI v Post-MI</th>
<th>Acute MI (1-3 months) v 1-7 days</th>
<th>Acute MI 1-7 days v 8-28 days</th>
<th>Acute MI 1-7 days v 1-3 months</th>
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</thead>
<tbody>
<tr>
<td>HNA clotting time</td>
<td>sec</td>
<td>*</td>
<td>&lt; 0-05</td>
<td>&lt; 0-001</td>
<td>*</td>
<td>NS</td>
<td>&lt; 0-10</td>
<td>&lt; 0-001</td>
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<tr>
<td>Platelet count</td>
<td>× 10^8/l</td>
<td>&lt; 0-01</td>
<td>NS</td>
<td>&lt; 0-1</td>
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<td>NS</td>
<td>&lt; 0-005</td>
<td>NS</td>
<td>&lt; 0-05</td>
</tr>
</tbody>
</table>

**Table Results: Mean ± 2 standard deviations**

*Heparin neutralizing activity: clotting time.

*From the figure these are seen to be profoundly different.

times of 28-2 seconds for patients with no history of MI and 20-9 seconds for post-MI patients. All these figures are significantly different from the others (see table), indeed the ac-MI results virtually represent a different population statistically.

It will be seen that 49 results form a remarkably tight cluster from 8 to 16 seconds while five results from 19 to 28 seconds lie in the range of the post-MI and normal figures. The diagnosis and results of these five were further scrutinized without indicating any difference; however, one of these five patients was studied serially and his clotting time on the third day after the episode was 12 seconds.

**Acute chest pain without myocardial infarction**

This group probably does not represent a single pathological state; thus perhaps it is not surprising to find that there is a wide scatter of results (fig 1 and table). Nevertheless it will be seen that 32 of the 34 results are over 16 seconds while 49 of the 54 patients with ac-MI gave results shorter than 16 seconds. Many of the patients with angina were studied serially and it is perhaps relevant that two of the shortest times (13 and 11 seconds), when repeated on the second and third days later, rose to 18 and 20 seconds. In both, the ultimate diagnosis was 'possible small pulmonary embolus'.

**Follow-up of acute myocardial infarction patients**

Most patients were studied several times, and with the exceptions noted above the results from day to day showed little variation. In 12 patients with ac-MI, figures were available until the follow-up at one to three months (fig 2 and table). It will be seen that there may be some changes in the tests by the second week, while by the time of the follow-up the results are similar to those of other post-MI patients in the larger study who were studied only at three months to five years after the infarct (O'Brien, 1974; O'Brien et al, 1974a; O'Brien et al, 1974b).

One patient failed to improve. Initially the HNA clotting time was 13 seconds and it never rose higher.
than 15 seconds. This patient had two further episodes of infarction and died three weeks later.

Studies prior to acute myocardial infarction
Two patients developed acute infarction while being studied because they had claudication. One was studied 24 and 17 days before the episode. He had very short HNA times (11 and 11 seconds) although he then had no cardiac symptoms. On admission after his infarct the time was 10 seconds. The second patient was studied once, two days before his infarct, when the HNA clotting time was 13 seconds. Unfortunately he was not followed up. The third patient was admitted with angina (HNA clotting time 20 seconds). Three days later he collapsed with a frank infarct and his HNA time 24 hours later was 11 seconds.

Other tests
The platelet count of the acute MI group when first studied (mean 189 000/µl) was insignificantly lower than that of the chest pain group (mean 216 000/µl). The count rose between 8 and 28 days (mean 272 000/µl) and then returned to the initial level by one to three months. (For the significance of these and other comparisons see table.)

The mean platelet volumes of both the patients with ac-MI (mean 9-64 fl) and those with chest pain (mean 9-65 fl) were similar and much larger than that of the control group (mean 8-00 fl).

Discussion
While many methods of measuring HNA have been published and a number of reports of abnormalities in ac-MI (Ogura et al, 1946; Rosenthal and Weaver, 1952; Gormsen, 1959; McDonald and Edgill, 1961; Holger-Madsen, 1962; Farbiszewski et al, 1968; Cotton et al, 1972) this appears to be the first systematic study of such a test applied clinically to all patients admitted with chest pain. These results indicate that our test for HNA is usually shorter in patients with ac-MI than in those with anginal type chest pain but without evidence of acute infarction. Thus, providing other causes for a very short HNA time can be excluded, for example, diffuse intravascular coagulation or venous thrombosis or pulmonary embolism, these findings strongly suggest that the HNA test can be used to confirm the presence of infarction. Clearly there are all degrees of myocardial ischaemia and all degrees of muscle death, and much more work will be needed to establish which of the available tests or combination of tests, namely, electrocardiogram or enzyme changes or, it can now be added, the HNA test will give the earliest and/or the most sensitive indication of muscle death. Nevertheless it can already be claimed that if other evidence is equivocal, a very short HNA time would suggest acute myocardial infarction and, conversely, if the HNA time is in the post-MI or normal range this would suggest the absence of significant muscle death.

Does this increase in HNA (short time) occur before the episode when it might be related to the cause or does it occur after the episode when it might be related to the result of the infarct? It seems unreasonable to suggest that all patients who subsequently develop MI should have long-standing grossly abnormal tests before the episode. At least by three months after the episode all those studied were shown to have the longer post-MI times. Thus we may accept that the HNA time becomes short in relation to the acute episode. In one of our patients studied days before the episode the HNA time was repeatedly shown to be very short. After he had recovered his post-MI time rose to the general run of post-MI HNA times. This might suggest an acute exacerbation of platelet activation before the episode. One other man also had a short time but a third man had an almost normal time of 20 seconds when studied some time before the infarct. Thus we have only fragmentary evidence of a pre-existing and possibly predisposing hyperactive platelet state. In any case the evolution of an infarct is certain to be different in different patients.

But, if, as seems probable, these very short times are normally found only immediately after the infarction—and this suggestion will be difficult to establish—then the excess HNA could be due to platelet factor 4 released by damaged platelets, for example, adherent to the dead muscle or released from platelets activated by thrombin formed in relation to the dead muscle. It is remarkable that the very short HNA times in acute MI change to the post-MI times in a few weeks, which is similar to the time thought necessary for healing of the infarcted area.

Since the HNA times are very short relative to the controls in ac-MI and are significantly shortened but to a lesser extent in post-MI and in atherosclerosis (O'Brien, 1974; O'Brien et al, 1974a; O'Brien et al, 1974b) it can be suggested that at least two separate processes in cardiovascular disease can be identified by this test. One is acute and transitory in ac-MI and perhaps related to cardiac muscle death, and the other is long-term and probably related to the degree of atherosclerosis. The test can probably be equally abnormal in other conditions, for example, in diffuse intravascular coagulation (O'Brien et al, 1974b; Fuster et al, 1974) and in acute venous thrombosis.

While the mean platelet count in the ac-MI group was a little lower than that in the chest pain group


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