Appearance of a Phosphorus Compound in Platelet-rich Plasma on Clotting

Previous experiments have shown that when clotting begins in human plasma containing platelets ('platelet-rich plasma'), adenosine triphosphate rapidly disappears from platelets with little or none of it appearing in the serum[1]. Other experiments suggested that in such plasma a phospholipoprotein is formed which is later broken down, and that this may be plasma thromboplastin[2].

Under the conditions used in those experiments fibrin appeared 5–10 min. after citrated plasma was recalcified. Since then a technique has been developed which leads to the formation of the clot within 2 min. With this technique the activity of thromboplastin in plasma reaches its maximum within 5 min. During that time there is not only a considerable breakdown of platelet adenosine triphosphate but also the appearance of a water-soluble phosphorus compound. This compound is the product of the breakdown of either phospholipid or phosphoprotein in the plasma.

To demonstrate this we made use of blood obtained from patients suffering from polycythæmia vera. The patients were being treated with phosphorus-32; some of this was incorporated into their plasma phospholipids and phosphoproteins which became radioactive. We observed the fate of this radioactivity during clotting. A representative experiment was as follows:

A male patient aged seventy-two was given 5 mc. of phosphorus-32 intravenously. After 48 hr., 200 ml. of blood was obtained from an arm vein and mixed with 4 ml. of 19 per cent (w/v) sodium citrate. The blood was centrifuged at 1,500*g* and 0–1° C. for 20 min. The plasma was removed and centrifuged at 22,000*g* for 15 min. to sediment the platelets. The supernatant platelet-free plasma (40 ml.) was dialysed at about 4° C., first against 3 l. Krebs–Ringer phosphate solution for 2 hr., and then for 18 hr. against 3 l. Krebs–Ringer bicarbonate solution modified so as to contain no phosphorus[2] and no glucose. The reason for dialysing against the phosphate solution was to reduce the specific activity of exchangeable phosphorus, and against the bicarbonate solution to reduce the concentrations of water-soluble phosphorus compounds in the plasma. After dialysis the concentration of inorganic phosphorus in plasma was 6·0 μgm./ml. and its specific activity was 0·6 counts/min. μgm. The specific activity of the plasma phospholipids was 14·7 and that of the plasma phosphoproteins 10·7 counts/min. μgm. phosphorus.

Samples (5 ml.) of dialysed plasma were incubated at 37° C. in glass tubes, the inner walls of which had been roughened by grinding. Platelets obtained from a healthy person were mixed with the plasma and 0·125 ml. *M* calcium chloride was added to each sample. The activity of thromboplastin was assayed at intervals of a few minutes. After 0, 1, 2, 4, 8 and 16 min., 1 ml. of 60 per cent (w/v) trichloracetic acid was added to a tube and thoroughly mixed with the clotting plasma. The tubes were cooled in ice and centrifuged at 0° C. for 5 min. at 22,000*g*. The clear supernatant solutions were decanted and retained. The precipitates were re-extracted with 5 ml. 10 per cent trichloracetic acid and the extracts were added to the first supernatants. The solutions were analysed for adenosine triphosphate by the firefly method[3]; and for inorganic phosphorus, organic phosphorus set free by *N* hydrochloric acid in 10 min. at 100° C. (10 min. phosphorus), and total phosphorus by the method of Berenblum and Chain[4]. The radioactivity of the phosphorus fractions was determined and expressed as counts/min. μgm. phosphorus.

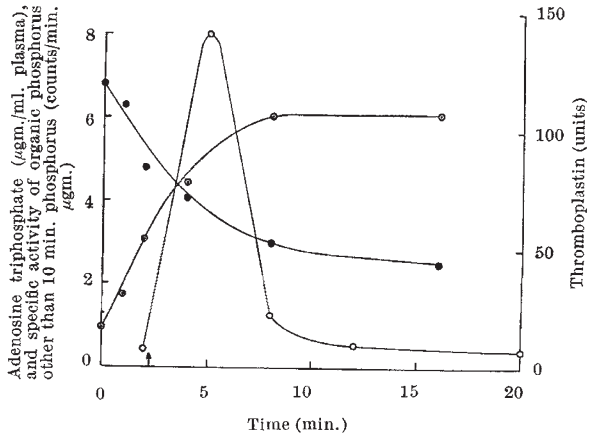

Fig. 1. Changes in the concentration of thromboplastin (○) and of adenosine triphosphate (●), and in the specific activity of organic phosphorus other than 10 min. phosphorus (⊙), in platelet-rich plasma during clotting. Calcium chloride was added at 0 min., and the clot formed after 2·3 min. as shown by the arrow

The results are shown in Fig. 1. The activity of thromboplastin increased steeply after 2 min., reached a maximum after 5 min. and then decreased almost equally rapidly. Adenosine triphosphate began to disappear within the first minute after the addition of calcium, and continued to do so at a decreasing rate for 16 min. At the time when the activity of thromboplastin was maximal almost half of the adenosine triphosphate had gone.

Also within the first minute there was an increase in the radioactivity of the trichloracetic acid solutions. This radioactivity continued to increase for about 8 min. and then remained constant. It was accounted for by an increase in the specific activity of organic phosphorus other than 10 min. phosphorus, which rose from 0·95 to 6·03 c./min. μgm. phosphorus. Since only the phospholipids and phosphoproteins had specific activities higher than this, the radioactive organic phosphorus which appeared in the trichloracetic acid solution must have originated from one or the other.

Five other experiments of this kind gave similar results. It may be concluded that, as thromboplastin is formed in plasma during clotting, plasma phospholipid or plasma phosphoprotein is broken down with the liberation of an acid-soluble substance containing phosphorus.

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