I. J. Med. Sc.
Seventh Series. Vol. 2. No. 5
May, 1969

FAILURE OF SURFACE POTENTIAL TO INFLUENCE THROMBUS FORMATION

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THIS communication is concerned with the ability of negative and positive electric potential on a platinum surface to induce or repel thrombus formation in the venous blood stream of the rat. It seems to show that surface potential has no effect on thrombus formation.

The thrombotic effect of positive charge was first described by Sawyer (1). Several writers (2-4) have used a positively charged electrode on blood vessel wall to induce thrombosis. In our experience this occurs only when the current is sufficiently strong (1mA or more) to damage the vessel wall, as described by Williams and Carey (2). There was evidence that small positive voltages (0.33/NHE) would precipitate platelets in vitro (5), and negative ones maintain the patency of metal prostheses in dog arteries (6, 7). However, the clotting time of human blood in metal cups is not related to the difference in potential between the two (8). It was decided to test the relationship between surface charge and thrombus deposition in vivo, using a standard procedure, and large numbers of observations. Due to the wire electrode used being relatively thick and obstructive in rat arteries, the vena cava was chosen as site. The circuit used was a modification of one developed in Dr. P. N. Sawyer's laboratory. One hundred and forty-seven preparations were examined.

A $1\frac{1}{4}''$ long wire*, .015″ in diameter, was inserted to lie free in the vena cava through a branch vein which was tied around the wire. It was connected to a 6 volt battery by means of a potential divider. The circuit was completed by a platinum plate 2 centimetres square under the skin in the animal's flank. The potential of the wire was measured by either an electrometer** having an impedence of 10^7 ohms with one input grounded, or by a double ended electrometer*** with 10^{14} ohms impedance. The double ended electrometer enabled ungrounded potential measurements to be made. These readings were consistently 10-12 mV higher than those taken with the grounded electrometer. A calomel half cell in a pool of saline in the abdomen was used as reference. By consistent measurement the pool of saline was at the same potential as the blood as measured by a calomel half-cell connected to the blood by a saline bridge. There was no measureable gradient across the vein wall. All voltages are referred to the hydrogen electrode.

Three series were run. In the first, platinum wires were used and for each 100 mV step in the voltage range +400 to −400 mV NHE, eight

*The wires were pure metals obtained from Johnson Mathey & Co., London.
**Radiometer pH meter model 23e Radiometer Ltd., Copenhagen.
***Keithley model 603 Electrometer amplifier. Keithley Instruments Inc., Cleveland, Ohio.

determinations were made. The platinum was brought to potentials varying from +600 mV to −500 NHE. It was impossible to bring the platinum wire more negative than −500 mV without electrolysis.

Before insertion, each wire was weighed to one-tenth of a milligram. On insertion, the potential was applied immediately. Observations were made on a pair of animals at a time, the conditions being similar in both. The wire was maintained at the chosen potential for 1 hour. Then the vena cava was clamped or tied proximally, and perfused with 10% formalin in Hank's solution at pH 7.2 and left for 5 minutes. It was then removed and slit open. The wire was freed and weighed wet. The thrombus weight was calculated. It varied in different animals at the same potential but showed no increase with positive potential so long as no electrolysis occurred (Fig. 1).

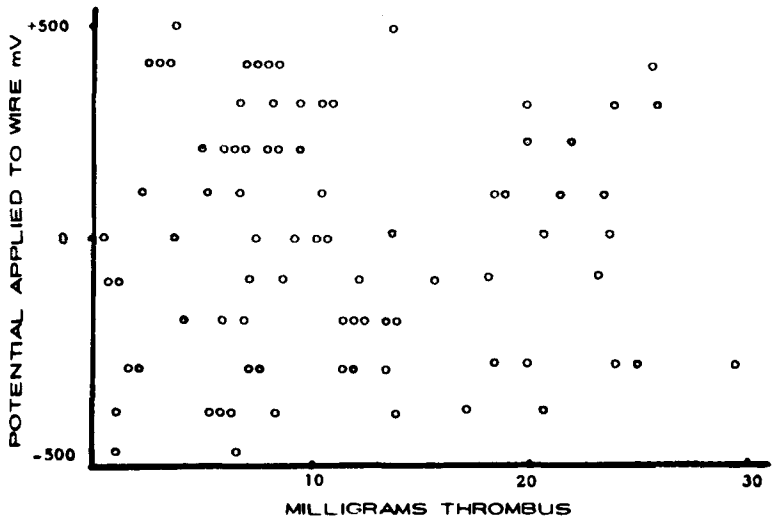

Fig. 1—Weight of thrombus forming on platinum wires plotted against their potential difference to the blood. They were set in the inferior vena cava of the rat for 1 hour.

The mean of the observations was 13.8121688 and the standard deviation 12.988821. Regression analysis carried out on the data, where y is the 'degree of thrombosis' and x the interface potential, showed the line to be $y=13.815139-0.002521x$, with slope 0.0025 and standard error of slope 0.0055.

These indicate that there is no evidence that the slope is other than zero, which means that the amount of thrombus forming on the wires is not related to their positive or negative potential.

There remained the possibility that thrombus might gather more rapidly on a surface that was positively charged. Eight rats had platinum wires inserted for periods of 5, 10, 15, 20, 25, 30, 40, 50 and 60 minutes respectively. All the wires were at a potential of 400 mV NHE positive to the blood. A duplicate series of animals bore wires charged negative to the

blood by 400 mV NHE. Thrombus weight in both series increased with duration of exposure in a roughly linear way (Fig. 2). Positive charge did not speed up thrombus formation.

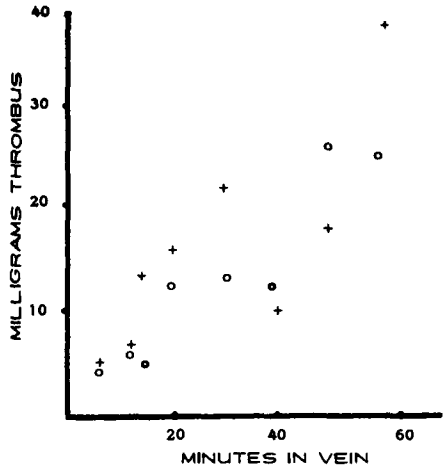

Fig. 2—Weight of thrombus forming on platinum wires plotted against the duration of their exposure to blood in the inferior vena cava of the rat. One series was charged at +400 mV NHE to the blood (o), the second at −400 mV NHE (+).

Due to a shortage of plantinum wire it was necessary to clean the pieces after use. Thirty-six were used in all. After removal of the thrombus, they were boiled in concentrated sodium hydroxide and washed in acetone, distilled water and saline. They were handled by forceps to avoid contamination by the fingers.

A third series was carried out using aluminium wire, whose spontaneous

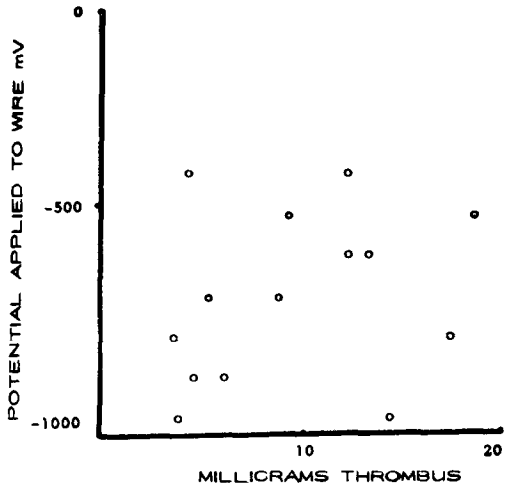

Fig. 3—Weight of thrombus forming on aluminium wires plotted against their potential difference to the blood. They were set in the inferior vena cava of the rat for 1 hour.

interface potential with blood is negative. In this series only two determinations were made at each step. It was found that at potentials positive to −400 mV NHE, electrolysis occurred. A voltage range of −400 mV to −1 volt was examined again in 100 mV steps. The results are similar to those obtained with platinum (Fig. 3).

Thrombus was found on the wires in all experiments. On both negative and positive wires it consisted mainly of platelets by electron microscopic examination. Out of a total of more than 140 preparations, only 1 mural thrombus was seen where the wire on insertion had damaged the vessel wall. The total thrombus weight (372.7 mgm.) on the 32 preparations that were positive to 0mV NHE was essentially the same as that (354.3 mgm.) on the 32 preparations that were symmetrically negative to 0mV NHE (Fig. 1).

Summary

Platinum wire was inserted in rat vena cava for 1 hour, and deposited thrombus was weighed. The wires were charged in 100 mV steps through the range ±400 mV NHE with respect to the blood. Eight preparations were used at each step. Observations were also made using aluminium wire in the range −400 to −1000 mV NHE. Inserted for various times, platinum wires charged at +400 mV NHE gathered thrombus at a constant rate over 60 minutes. Wires with a negative charge of 400 mV gathered thrombus at a similar rate.

The results indicate with statistical confidence that surface charge does not affect the rate or amount of thrombus deposition on metal surfaces.

It would seem from these results that there is no relationship between the electrical potential of a charged wire in the rat vena cava and the amount of thrombus it gathers during the first hour. It may be that rat venous blood reacts in a way different to that of dog arterial blood.

This work was supported by the Medical Research Council of Ireland.

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