## SCIENTIFIC APPARATUS AND LABORATORY METHODS

## THE BLOOD-SALINE COAGULATION TIME TEST

THE coagulation time of whole blood is influenced by mechanical agitation, temperature, foreign surfaces, air bubbles and the size and shape of containers in relation to blood volume. All coagulation time tests do not yield comparable readings because of these variable factors. The introduction of a new coagulation time test is justifiable only if it offers significant advantages over the multitude of methods available. We have developed a method which is based on the phenomenon, which we studied that whole blood diluted with physiologic saline delays the coagulation time. This method is subject to the same variable influences listed above. Furthermore, we found that a relation exists between the final concentration of blood in physiologic saline and the coagulation time, the latter becoming more prolonged in lower blood concentrations.

Four Pyrex glass tubes, 75×10 mm, are used in each test. One cc of physiologic saline is added to three of these tubes. Ten cc of blood are withdrawn from the antecubital vein and the needle removed. Since tissue juice, which may enter the syringe initially, affects the coagulation time, only the 3 or 4 cc of blood which enter the syringe last are expelled into a large tube. From this tube 1 cc is then pipetted into the first tube which does not contain saline. Another 1 cc is pipetted into the second tube containing saline and aspirated well 3 times, care being taken to avoid the formation of bubbles. From this mixture 1 cc is pipetted into the third tube and the process of serial dilution carried out through the fourth tube from which 1 cc is discarded. The average time from the moment that the blood entered the syringe until the last dilution was made was found to be 92 seconds in 54 men, the longest interval being 115 seconds. The starting time was arbitrarily adopted as the time the blood first entered the syringe. It is important that all 4 tubes be tilted in the same manner and at the same interval of time. Therefore, as a rule, readings should be done every 30 seconds up to 15 minutes, and afterwards every 5 minutes until all samples have clotted. These reading times can be modified whenever hyper- or hypocoagulable blood is tested. Since it is known that changes in temperature can markedly alter the coagulation time, tests should be run at constant temperatures, preferably. We chose as the endpoint the appearance of a gel. In most cases this gel was firmly attached to the glass wall. However, in a number of instances, a sliding

<sup>1</sup> J. J. Lalich and A. L. Copley, Proc. Soc. Exp. Biol. Med., 51: 232, 1942.

gel was formed in the tube containing the 12.5 per cent. blood concentration.

Table 1 gives the coagulation times obtained on fiftyfour medical students and members of the faculty.

TABLE 1
BLOOD-SALINE COAGULATION TIMES IN FIFTY-FOUR HEALTHY MEN

Blood concentration Per cent.	Time in minutes at 33°C.	
	Range	Average
100	3.0- 6.5	4.5
50 25	$\begin{array}{ccc} 4.0-&10.5\\ 5.5-&13.5 \end{array}$	$\begin{array}{c} 5.5 \\ 9.0 \end{array}$
12.5	14.0-365.0	70.0

No patients with hemorrhagic diseases were available. One interesting observation noted was that in the 100 per cent. and 50 per cent. blood concentrations the coagulation times were approximately the same. However, the 50 per cent. blood samples frequently showed a slightly increased coagulation time. Perhaps the degree of coagulability may prove to be more properly evaluated in blood concentrations of 12.5 per cent.

This coagulation time test was applied to studies of dogs2 in which hypocoagulability and hypercoagulability of blood had been produced experimentally. It was found that, in certain cases, hypercoagulability and hypocoagulability could be detected in the diluted blood samples, but not in undiluted blood. Hypercoagulability was indicated by shortened coagulation times in all blood concentrations. Hypocoagulability was found in the 50 per cent. blood samples. These exhibited markedly prolonged coagulation times, whereas the coagulation time of the undiluted blood was well within normal limits. These phenomena promise to reveal changes in the coagulability of whole blood which are not detectable by the present methods. In addition, it is significant that this principle of blood-saline dilution provides a new and simple method of isolating corpuscles and plasma constituents from whole blood without the use of any anticoagulants. Certain phases of this problem are under investigation.

> ALFRED LEWIN COPLEY RALPH B. HOULIHAN

SCHOOL OF MEDICINE, UNIVERSITY OF VIRGINIA

## A CONSTANT VACUUM APPARATUS

FREQUENTLY there is need in the laboratory for a device for regulating pressure derived from a house vacuum source to a constant level. An effective ap-

<sup>2</sup> A. L. Copley. To be published.