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 Ten cc of blood are withdrawn from the antecubital vein and the needle removed.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,[1] 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 gel was formed in the tube containing the 12.5 per cent. blood concentration.

Table 1 gives the coagulation times obtained on fifty-four 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	4.0– 10.5	5.5
25	5.5– 13.5	9.0
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 dogs[2] 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.

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[1] J. J. Lalich and A. L. Copley, *Proc. Soc. Exp. Biol. Med.*, 51: 232, 1942.

[2] A. L. Copley. To be published.

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-

paratus which can be constructed simply and inexpensively from odds and ends is illustrated in Fig. 1.

The instrument is constructed on a base of ¾ inch boards. The base is 19½ inches long, 4 inches wide and 3½ inches high. Regulation of pressure is accomplished by the movement of the oiled plunger of a 20 cc syringe mounted horizontally as shown. The head of the plunger is attached by means of a bridle to the coil spring. The other end of the spring is fastened in turn to a rack and gear that can be moved to and fro through the influence of the knurled screw. Included in the bridle is the upper end of a metal lever passing down through a ½ by ⅞ inch hole in the top of the base and fastened with a hinge at the other end, as shown. A tube of resilient rubber 3/16 inch internal diameter and 3/32 inch wall thickness is passed between the lever and the perpendicular block and fastened with metal loops screwed to the block. The rubber tube continues and joins a T tube of glass, one limb of which communicates by way of a short piece of rubber tubing to the syringe. The third limb of the T tube communicates distally with other apparatus where the constant vacuum is desired.

In operation, the spring pulls out the plunger, and at the same time pulls the lever away from the rubber tube leading from the house vacuum source. With the vacuum turned on, the full pressure is exerted throughout the system, including the syringe. When flow of air is interrupted in the distal parts of the system, the pressure falls inside the syringe, sucking in the plunger to an extent proportional to the degree of interruption of flow. When the plunger moves forward, so, too, does the lever, compressing the rubber tube between it and the perpendicular block. The air flow is then interrupted at this point to a degree depending on the tension of the spring and is stopped completely if tube to the apparatus is closed off. The interfering resiliency of the rubber tube is counteracted by the auxiliary second spring attached to the lever beneath the top board.

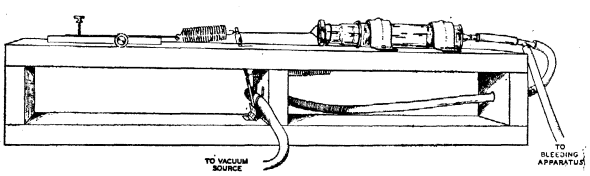

FIG. 1.

An example of the use of the device is in connection with the bleeding of chickens (or other small animals) from the heart into citrate solution. A special arrangement of syringe and needle for this purpose is shown in Fig. 2. For this a 50 cc syringe is fitted with a rubber stopper with two bends. One communicates through a short rubber tube and a short glass air jet (.010 inch diameter) with the long rubber tubing leading to the constant pressure device. The second bend reaches down the outside of the syringe to a sharp angle and the open end, which is glazed smooth. To the end of the syringe is fitted an adapter, into which a section of the shaft of a No. 16 gauge needle has been soldered, providing a means for holding the citrate solution in the syringe. For bleeding chickens a No. 18 gauge needle is fitted to the adapter.

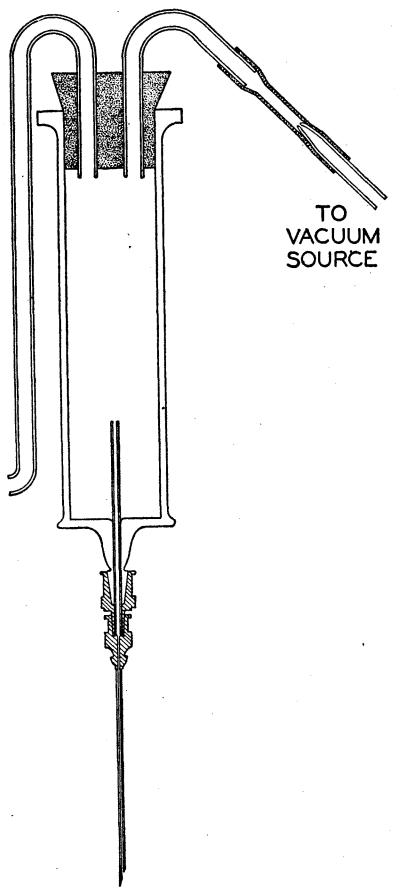

FIG. 2.

To use the device, the vacuum, regulated by the instrument of Fig. 1, is turned on. The needle is dipped into citrate solution and, with the index finger closing the open end of the downward bend, citrate solution is drawn into the syringe to a level somewhat below that of the needle projecting into the syringe. Then, with the end of the downward bend open, the needle is manipulated into the heart. When the blood shows, the opening of the downward bend is again closed with the finger, the vacuum at constant low pressure is exerted and the blood is drawn into the syringe.

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