TABLE II.
Effect of Initial Swelling on Hemolysis Time.

| Time of exposure in hr | Time in seconds for 50% hemolysis | | Cell volume in $\mu^3$ | |
|---|---|---|---|---|
| | Plasma | 80% R.L. | Plasma | 80% R.L. |
| 0 | 345 | 350 | 112 | 128 |
| 20 | 200 | 230 | 126 | 139 |
| 0 | 365 | 370 | 123 | 132 |
| 23¼ | 190 | 220 | 136 | 139 |
| 0 | 340 | 340 | 125 | 143 |
| 24 | 205 | 240 | 146 | 160 |

tions change so markedly indicates additional factors which must be taken into consideration in hemolysis experiments. No satisfactory explanation is available at present for the apparent lack of effect of a slightly hypotonic environment on hemolysis time.

In summary it may be said that control chicken erythrocytes after standing at 37.5°C for several hours hemolyze more rapidly. An increase in the volume of these cells was observed but this cannot be the sole explanation for the change in rate of hemolysis.

15862

## A Simple Inexpensive Pump for Perfusion of Organs with Preservatives or with Physiological Solutions.*

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In an attempt to investigate the secretory properties of human testes by means of perfusion *in vitro*, 3 types of perfusion pumps were employed but none were found to be suitable for these purposes. The Carrel-Lindbergh pump[1] proved to be so readily subject to disturbances upon change in the resistance offered by the organs during perfusion that maintenance of a proper flow of blood was difficult. The pump devised by Kleinberg and associates[2] is less complex, but in our experience the valves were prone to stick, and during long experiments trouble was encountered in stabilizing the flow of blood through the shunt and in preventing changes in the level of fluid in the compression chamber. The Fischer "Volustat"† came nearest to filling the requirements of our experiment but afforded no means for regulation of the rate of pulsation.

Study of the mechanical principles of these and other pumps, *e.g.*, the Dale and Schuster apparatus,[3] suggested the need for a pump with the following characteristics: (a) ready construction at small cost; (b) sufficient simplicity in operation as to provide rapid and satisfactory perfusion of an organ with physiological solutions or with preservative fluids; (c) no need of continuous adjustment during prolonged periods of perfusion; (d) the capacity to mimic the action of the heart by providing any desired rate of pulsation

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1 Carrel, A., and Lindbergh, C., *The Culture of Organs*, Paul B. Hoeber, Inc., New York, 1938.

2 Kleinberg, W., Gordon, A., and Charipper, H., *J. Lab. Clin. Med.*, 1943, **28**, 1484.

† Fischer, "Volustat," Eimer and Amend catalogue 90, No. 13-684.

3 Dale, H., and Schuster, E., *J. Physiol.*, 1928, **64**, 356.

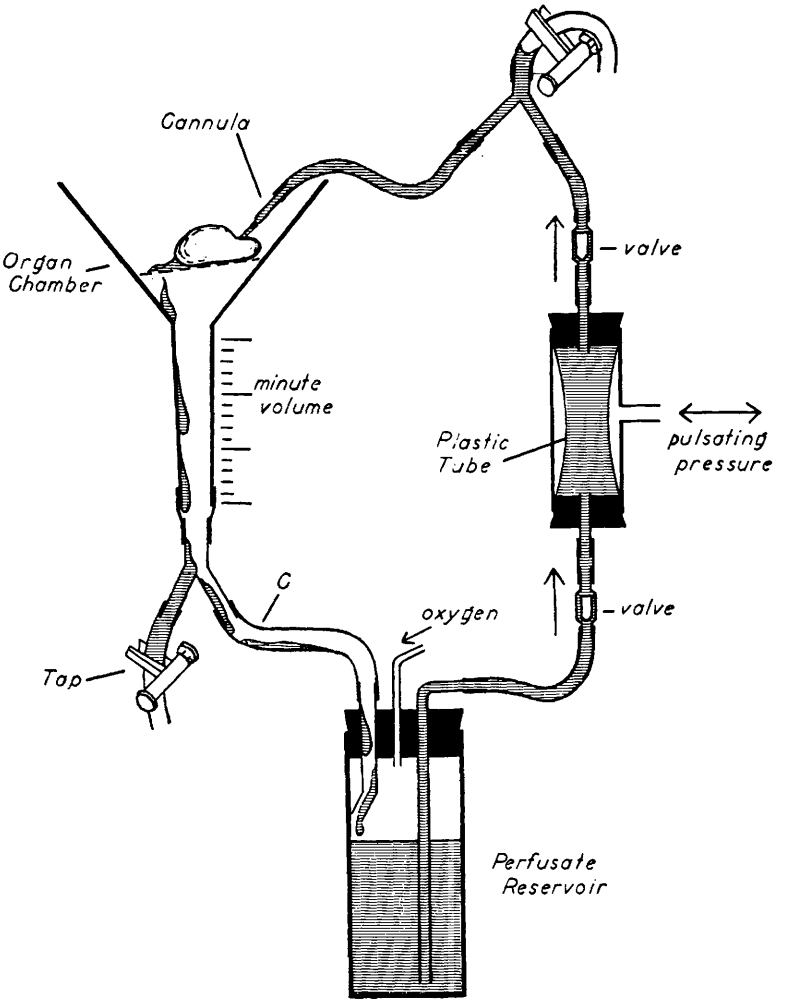


Fig. 1

and volume of fluid per pulsation; (e) allowance for the necessary degree of oxygenation of the perfusate; (f) the ability to use perfusates either in large quantities or amounts as small as 25 cc; (g) facilities for addition or removal of aliquots of the perfusate while the pump is in operation; (h) construction that permits easy determination of minute volume; (i) composition of materials that are easily sterilized and not toxic to tissues.

*Description of Apparatus.* The final design of the pump (Fig. 1) is the serial successor of many and was arrived at by a process of trial and error. Its essential parts are the organ chamber which is a funnel, the perfusate reservoir, the pump proper including its 2 valves, and tubing connecting these 3 parts.

The pump proper is a simple device made from a Pyrex test tube, 200 x 25 mm, with a side tube. The closed end of the test tube was removed. A piece of plastic tubing was placed inside the glass tube and pulled over each open end in such a way that when stoppers were inserted into the ends of these plastic and glass tubes the stretched plastic was held firmly in place. By interposing this between 2 valves directed as indicated in Fig. 1 and by supplying a pulsating pressure of air to the side tube, the pump becomes complete.

A positive thrust of air compresses the plastic tube and causes the lower valve to

close while the upper valve is opened; release of the positive thrust permits the elastic plastic tubing to expand and so causes the lower valve to open while the upper valve is closed. Fluid is thus pumped from the reservoir to the cannulated organ.

As stated above, the funnel represents the chamber in which the organ rests on a glass plate. From this the perfusate drips into the stem of the funnel. The inverted Y-tube attached to the stem makes it possible to deflect the perfusate into either the perfusate reservoir or the tube labelled "tap" in the illustration. With a clamp on the tap and a second clamp at the point "C" the minute volume can be estimated from the time required to fill the graduated funnel stem. Samples of perfusate may be obtained for analysis by removing the clamp from the tap.

The perfusate reservoir receives the fluid returning from the organ being perfused and also a tube bearing oxygen or any mixture of gases. If desired, the oxygen may be bubbled through the perfusate before it passes into the tube and up through the funnel stem to be dissipated. As the oxygen passes toward the organ chamber it contacts the descending film of perfusate.

From the perfusate reservoir the fluid is pumped into the organ thus completing the circuit.

Bubbles of gas may pass through the pump proper toward the cannula, when the fluid first flows through the apparatus or if the reservoir is agitated. An inverted Y-tube with a clamp guarding the outlet acts as an adequate trap to eliminate air emboli which might be pumped into the organ. The pressure in the system may also be measured at this site.

The pump receives a pulsating pressure of air, *i.e.*, a regular positive thrust alternating with reduction of pressure to that of the general atmosphere. Because a Carrel-Lindbergh apparatus was available we used the pulsating valve made at the Rockefeller Institute and driven by a 1/30 H.P. motor supplied by the Janette Manufacturing Company of Chicago. This motor is equipped with reduction gears and rheostat. Supplementing it one must have a source of compressed air. The pulsating valve and compressed air unit of the Carrel-Lindbergh apparatus are a luxury rather than a necessity. We have used successfully a heavy-walled rubber bulb compressed by a motor-driven adjustable plunger. The Wiggers' artificial circulation machine[4] used in many teaching laboratories has such a rubber bulb and works well with this apparatus.

The flexible plastic tube which receives the pulsating pressure has an inside diameter of 11/16 inch. The inside diameter of tubing used elsewhere was 5/32 inch except for the piece marked "C" in Fig. 1 which was 3/8 inch. The tubing is furnished by the Industrial Synthetics Corporation of 60 Woolsey Street, Irvington, N.J., under the tradename of Voltron.

The glass valves were obtained from an old-fashioned 2-way syringe used for blood transfusions. Glass ball-valves are also suitable. If the requirements of the perfusion are not too exacting, valves from a sphygmomanometer bulb may be employed.

*Merits of the Apparatus.* The apparatus can be assembled in less than one day's time at a cost of about 5 dollars. It is easy to operate. During perfusion of human testes and rat kidneys for as long as 8 hours minor adjustments were made only at intervals of an hour or more.

The stroke volume and rate of beating of the heart can be simulated over a wide range. Systolic pressure can be as high as 300 mm of mercury with a drop to zero in the diastolic phase when no organ is providing resistance. The number of pulsations per minute can be varied from 85 to 122 with the Carrel-Lindbergh pulsation valve.

While the perfusion is in progress aliquots of the perfusate may be removed or more added, thus allowing serial study of changes in the perfusate or trials of entirely different media. Pumps of various sizes might be employed. The present apparatus works with a volume as small as 25 cc but quanti-

[4] Wiggers, C. J., *Physiology in Health and Disease*, 4th edition, Lea and Febiger Co., Philadelphia, p. 643, 1944.

ties as small as 5 to 10 cc could probably be used if the apparatus were produced in miniature. This would permit perfusion of an organ of a small rodent with blood from the same animal.

The plastic tubing imparts mobility to the apparatus and reduces the danger of breakage. In addition this tubing is nontoxic for organs as judged by its effect upon tissue cultured in close association with it. To test this fragments of 8-day chick embryo heart ventricles were planted in equal parts of blood plasma and embryo extract. Before clotting occurred 3 chips of tubing were set close to each tissue fragment so that if growth occurred it would be over and around the plastic. Of several plastics tested, "Voltron" was the only one which had no apparent effects over an observation period of 3 days. Preliminary to cultivating the tubing was sterilized by immersion in 70% alcohol for 24 hours and the alcohol was removed by immersion in sterile Tyrode solution.

*Limitations of the Apparatus.* Among the more serious limitations of the apparatus is the fact that when whole blood is used in considerable quantity the corpuscles settle in the reservoir. A means for agitating the fluid in the reservoir would be a desirable addition to this apparatus.

For sterile procedures the funnel has to be replaced by a more elaborate organ chamber. Suitable chambers have a drainage tube and an entrance for a 2-hole stopper. One opening in the stopper admits the cannula, the other a cotton filter through which escapes the oxygen which passed to the organ chamber from the reservoir.

The apparatus contains no filters. For non-sterile work we set a small pad of cotton over the neck of the funnel. If needed, more elaborate sand filters can be employed.

Still other refinements might be adopted, but the pump has proved to be satisfactory as it is. For example, an entire rat can be rendered blue by fluids containing various amounts of trypan blue. As another illustration, microscopic examination of fresh spreads or sections of tissues from a rat perfused with Ranvier's gelatin carmine showed complete injection of the vascular trees in the intestines, kidneys, liver, and other organs. Many but not all of the vessels in the skeletal muscles appear to be filled.

*Summary.* Description is given of an easily constructed perfusion pump and of the satisfactory results obtained with its use.

A type of plastic tubing used in this pump was found to have no discernible toxic effects in tissues cultured on it *in vitro*.

## 15863

## Mitotic Response to Colchicine in Human Cancer.*†

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Colchicine, the alkaloid isolated from the corm and seed of *Colchicum autumnale* L, has been known to arrest nuclear divisions in the metaphase stage in generative tissue of plants and animals. During the past 15 years the studies with colchicine initiated by Dustin, his collaborators and students have been amply tested, and frequently reviewed (Levine[1] and Ludford[2]). Here, only references pertinent to the problem under investigation will be made.

Colchicine and about 47 other chemical

* The aid rendered by the Social Service Bureau, Knights of Pythias, is gratefully acknowledged.

† We are indebted to S. B. Penick and Co. through the courtesy of Dr. George M. Hocking for some of the colchicine used in this work.

1 Levine, M., *Bot. Rev.*, 1945, **11**, 145.

2 Ludford, R. J., *J. Nat. Cancer Inst.*, 1945, **6**, 89.