Micro respirometry studies, with the aid of the Cartesian diver (1, 2), have presented several problems in manipulation. One of these has been the problem of mixing two or more solutions quantitatively in the diver, after the start of an experiment. Methods have been described accomplishing this (3, 4). These methods could be mastered only by special training, patience, and skill.

A much less difficult technique is now proposed in which a new type of diver vessel is employed, having an upper reaction chamber, a lower expansion chamber, and a relatively long neck. Precise control of overpressure necessary to mix the solutions in the diver is accomplished by the use of a sphygmomanometer bulb and valve, connected into the manifold system by a 3-way stop-cock (Fig. 1). The inner surfaces of the diver need to be coated with a hydrophobic surface such as Clarite, dissolved in toluene, only once. Thereafter there seems to be enough residual coating to take care of the requirements. Sudan III is mixed with the paraffin oil to facilitate visual observation of its position in the diver neck and phenolphthalein is used in the NaOH to detect any changes in pH.

**Hour-Glass Type of Diver**

A method for making Cartesian divers has been described (Claff (5)), in which the various steps are carried out in a jig. The preliminary steps in the process of making the new type diver vessel are the same as for the conventional diver. The "blank" is carried through all steps, including Steps E, F, and G (see (5), Fig. 1). The capillary tubing is now as shown in Fig. 2, A. At this point the flame is placed in position x on the capillary, at least 4 to 6 mm. to the right of the bubble just formed. The capillary is twirled to and fro and a second bubble is formed. The end result should be as shown in Fig. 2, B.

To achieve this result it is necessary to start heating the capillary at least 4 to 6 mm. from the first bubble. The reason is easy to follow. The first bubble was formed from part of the glass from the solid molten mass of the tail. The second bubble has no such source of glass and the glass must come from the capillary tubing itself. If the second bubble is
started too close to the first bubble, it simply coalesces with it, and one very large bubble is formed instead of two small ones.

To complete the diver, it is necessary to cut the capillary so that the neck is at least 15 mm. long (Fig. 2, B), calibrate it, and add glass to the tail.

![Diagram of the modified Cartesian Diver system](http://www.jbc.org)

**Fig. 1**

*Specifications of Hour-Glass Type Diver (Fig. 2, C)*—The following specifications are desired for this type of diver: capillary tube, 1.22 mm. outside diameter (0.048 inch); neck length, 15 mm.; bubble diameter, 3 mm.; volume, 30 to 34 c.mm.; and total weight, approximately 97 mg. Suggested fillings are 2 c.mm. of oil, Solution 1, Solution 2, or alkali. The oil seal should be at least 2 c.mm. The other fillings are arbitrary, but must
be predetermined before calibrating the diver, for which the procedure is the same as for the conventional diver (5).

_Filling the Diver—_The alkali drop, if used, is deposited by a calibrated pipette held in a diver filler device, as are Solution 2 and the oil seal. Solution 1 is placed on the side of the reaction chamber by the use of the "braking pipette" described by one of us (C. L. C. (6)). The pipette should have a straight sided tip at least 25 mm. long, coated with Clarite, paraffin, or lanolin or some other hydrophobic surface.

_Fig. 2. Cartesian diver jig and new type of diver vessel to facilitate "mixing"_

**EXPERIMENTAL**

To test the efficacy of this method the following experiments were performed. A suspension of _Arbacia_ eggs was placed in the reaction chamber (Solution 1); a suspension of _Arbacia_ sperm was placed in the lower end of the neck (Solution 2). An oil seal was placed in the upper portion of the neck, and an alkali drop at the bottom of the expansion chamber. While the diver vessel was observed through the microscope, mounted on the bath, the pressure of the manifold was slowly increased by the use of the sphygmomanometer bulb; the sperm suspension was slowly forced into the reaction chamber, where it coalesced with the egg suspension. As soon as the drops coalesced, the pressure was slowly returned to its initial pressure, by means of the valve on the sphygmomanometer bulb.

Subsequently, cleavage was observed, proving the eggs were fertilized. The accuracy of the entire system, including the calibration of the pipettes used, was tested by loading the reaction chamber with 2 c.mm. of 0.01 M...
NaHCO₃, the lower portion of the neck with 2 c.mm. of 1.0 N H₂SO₄, and the seal with 3 c.mm. of oil.

After a short equilibration period, overpressure was applied until the solutions were mixed, and then slowly and evenly the pressure was returned to the original pressure by releasing the valve on the sphygmomanometer bulb.

The CO₂ evolution was recorded for 20 minutes. The average of six experiments showed a recorded evolution of 97.0 per cent of the theoretical yield of CO₂. One experiment with 1 c.mm. of 0.01 M NaHCO₃ and 2 c.mm. of 1.0 N H₂SO₄ showed a recorded evolution of CO₂ equal to 97.3 per cent of the theoretical yield (Fig. 3).

Since the contents of the diver may be readily examined at will by the aid of the microscope mounted on the bath, it is possible to correlate the pattern of the observed behavior and morphological changes of the material contained in the diver with the respiratory rate recorded.

This was shown by the following experiment with Paramecium cahinski, Mating Types I and II.

The diver vessel was loaded with Mating Type I in the reaction chamber and Mating Type II directly above in the lower portion of the neck.

One diver was used as a control and the contents were not mixed. After the combined respiration of Mating Types I and II was recorded for 50 minutes in each diver, the contents of the experimental diver were
mixed. A reduction of respiration rate in the experimental diver was recorded for the next half hour; then a partial return to the former rate of respiration occurred. It was possible to observe the initial clumping of the *Paramecium* within 30 seconds of the mixing. This we could correlate with the depressed respiration. After half an hour we observed con-
as follows (Fig. 5). The *Chaos chaos* were washed several times and allowed to become acclimated in acetate buffer, pH 6.6, made up with boiled tap water.

**Fig. 5.** Inhibition of respiration rate in *Chaos chaos* due to addition of uranyl nitrate, and recovery upon addition of citrate and phosphate.

The control diver was loaded with seven *Chaos chaos*, 0.1 N NaOH 2 c.mm. of uranyl nitrate, and an oil seal. The contents of this diver were never mixed. Its respiration rate is shown on the control graph (Fig. 5). The experimental diver was loaded in the same manner, and the respiration was recorded for 1 hour. The uranyl nitrate was then mixed with the drop containing the seven *Chaos chaos*. Respiration was inhibited 20 per cent.
After the 2nd hour the experimental diver was recovered from the bath, the oil seal removed, the neck of the diver cleaned with a spill of filter paper, and a charge of citrate and phosphate buffer (pH 6.4) was placed in the lower portion of the neck of the diver, together with a new oil seal. After a short equilibration period, the citrate-phosphate buffer was mixed with the drop containing the uranyl nitrate-treated *Chaos chaos*. Through the formation of the uranyl citrate complex, the toxic effect of the salt was eliminated, and a respiration recovery of 100 per cent was recorded.

We wish to acknowledge our indebtedness to Dr. E. S. Guzman Barron for his interest and suggestions during the course of our experiments. We also wish to thank Dr. A. A. Schaeffer for furnishing the cultures of *Chaos chaos*, and Dr. Ralph Wichterman for cultures of *Paramecium calkinsi*, Mating Types I and II.

**SUMMARY**

A technique is described, with a new hour-glass type of Cartesian diver vessel, which makes it relatively easy to mix quantitatively one or more solutions in the diver vessel at any time during an experiment. Some results and suggested uses of the method are described.

**BIBLIOGRAPHY**

CARTESIAN DIVER TECHNIQUE
C. Lloyd Claff and Theodore N. Tahmisian

J. Biol. Chem. 1949, 179:577-583.

Access the most updated version of this article at http://www.jbc.org/content/179/2/577.citation

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/179/2/577.citation.full.html#ref-list-1