

intakes, though low, were not reduced to the minima. Repeatedly we<sup>2, 6, 7</sup> have pointed out that mixtures of purified amino acids, compounded in accordance with the quantitative needs of the cells for each component, may prove to be the most efficient type of nitrogen ever devised for the uses of the animal organism. For some time investigations have been in progress<sup>7</sup> to establish the lowest intakes of such preparations which are capable of maintaining nitrogen equilibrium in the rat and in the dog. The results will be reported later.

Inasmuch as the successful use of synthetic mixtures of amino acids in nutrition studies was made possible by the discovery of threonine in this laboratory, it seems not inappropriate to expect that a reasonable period of time will be allowed for the consummation of the program outlined above before similar studies are undertaken by others.

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## SCIENTIFIC APPARATUS AND LABORATORY METHODS

### DEEP-SEA PHOTOGRAPHY

INTEREST in deep-sea animals had led me to assemble an automatic camera mechanism in a pressure chamber<sup>1</sup> capable of withstanding two miles of depth in the sea, two tons per square inch, with a considerable safety factor. In this self-contained device, two six-volt storage batteries supply the current to run the motor (12 volts) for a 16 mm moving picture camera, a 50 candle power headlight with reflector (8 volts), and a timing motor (4 volts). The light shines through one "herculite" glass window, while the camera takes pictures through another. A pressure gage with electric contacts, which can be set for any depth, activates the mechanism by means of a lock relay. This starts the timing motor whose contacts turn on the movie camera and light (each through a separate relay) for 1.2 second and then turn them off for 11.1 seconds, when the process is repeated. The camera is set to take 16 pictures a second, and the films when developed show 20 light frames between 3 dark ones, since the filament takes some time to reach incandescence and the motor some time to stop. In 100 feet of film, there are about 170 chances of photographing something. Since the pictures are taken in the zone of perpetual darkness, a lure is hung 4 feet in front of the pressure chamber and the camera with stop f 1.5 focused on it. This lure is a wooden fish resembling a deep-sea fish, with rows of photophores painted on it with self-luminous zinc sulfide paint.

In June, through the kindness of Dr. J. F. G. Wheeler, of the Bermuda Biological Station for Research, I had the opportunity of testing the camera, which was let down from the ketch, *Culver*, permanently stationed in Bermuda for oceanographic work under the auspices of the Royal Society of London. Five descents were successfully accomplished in the

region 5 to 10 miles southeast of Bermuda, where Beebe<sup>2</sup> has made over 1,500 hauls with nets and many descents with the bathysphere. Three 100-ft rolls of super XX panchromatic film were taken at 500 fathoms, one at 800 fathoms, and one at 1,320 fathoms (1½ miles). In the latter, the chamber touched bottom (although the chart indicated plenty of depth), knocking off a support and turning the camera out of position so that nothing appeared on this film. The other four films showed the lure clearly but no fish or large organisms. However, 17 small creatures, the largest about one centimeter in diameter, too small to be identified, moved across the beam of light in the 300 feet of film taken at 500 fathoms, the depth where Beebe obtained most material in his hauls with the nets. The film at 700 fathoms only showed two small creatures.

Since the lens angle of the camera subtended a rectangle 8×11 inches at 2.5 feet and 20.5×26 inches at 6 feet, the depth of focus for f 1.5 stop, we can think of the camera as sampling a frustum of about 7 cubic feet or one fifth of a cubic meter. Because of the drift of the boat, 510 samples were made in the three films exposed at 500 fathoms and 17 organisms photographed. This is one medium-sized organism per 30 samples or 210 cubic feet (6 cubic meters) of sea.

While no striking photos were obtained of deep-sea fish attacking the lure or one another, the experiments show that deep-sea photography is quite feasible and might be developed into a method of estimating the density of organisms at different depths.

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### A GLASS ELECTRODE VESSEL FOR THE DETERMINATION OF BLOOD pH

THE Beckman pH meter may be employed effectively in estimating the pH of whole blood under anaerobic conditions through the use of a special glass electrode assembly. The determination can be made directly on

Butt, research assistant in physiology, I am grateful for skilful arrangement of the wiring mechanism.

<sup>2</sup> Wm. Beebe, "Half Mile Down," Harcourt, Brace and Company, New York, 1934.

<sup>6</sup> W. C. Rose, *The Harvey Lectures*, 30: 49, 1934-35.

<sup>7</sup> W. C. Rose, *Proc. Inst. Med. of Chicago*, 12: 98, 1938.

<sup>1</sup> It gives me great pleasure to acknowledge the advice of Dean Greene and Dr. Moody, of the Engineering Department of Princeton University, in the design of the pressure chamber, which was a most acceptable gift of Mr. Owsley Brown, president of the Springfield Boiler Company, manufacturers of the chamber. To Mr. Charles

the blood within 40 to 50 seconds after collection. In a series of determinations made upon dogs, we have obtained consistent and readily reproducible results.

A small glass vessel, illustrated in the figure, was made, having two side arms. This vessel may be attached to the standard Beckman glass electrode by winding firmly with thin rubber dam and fastening with rubber cement. After a little practice an airtight connection can readily be made. If the Beckman electrode with the ground glass collar is available, the

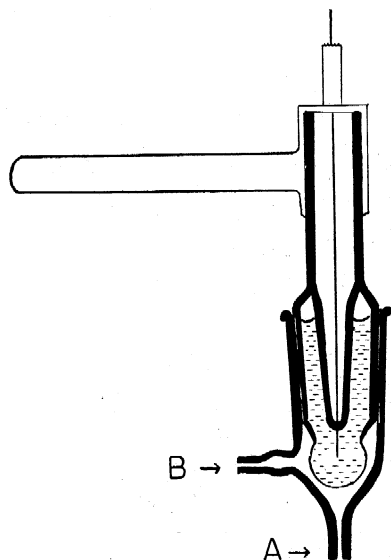


FIG. 1

vessel may be ground to fit this collar. The ground glass connection permits of more rapid adjustment and is more convenient for washing, but we have found the rubber connection to be perfectly satisfactory. The side arm, A, is attached by the shortest possible length of rubber tubing to a syringe adapter. If desired, this side arm may be made of a syringe tip, sealed into the cup, thus making a direct connection for the needle.<sup>1</sup> A ten-inch length of 3 mm rubber tubing attached to a mouthpiece is fastened to side arm, B, to be used in filling the vessel. The total capacity of such a chamber is 0.3-0.4 cc.

The procedure for performing a blood pH determination is as follows: Saturated KCl solution is warmed to 40° C. and placed in a small wide-mouthed vessel of about 50 cc capacity. This is supported so that the calomel half-cell dips into it and a thermometer is immersed in the solution, which serves as a bath and a salt bridge. Sufficient 1 per cent. neutral oxalate (pH 7.0) at 38° C. is drawn through A into the electrode vessel and a small clamp placed on the rubber tubing near the mouthpiece. A venipuncture is made with a

hypodermic needle and when a constant flow of blood is obtained, the chamber is attached to the needle and the blood drawn into the vessel by opening the clamp and exerting suction on the mouthpiece. From 0.5-0.7 cc of blood is drawn into the vessel and tubing to insure complete replacement of the oxalate. It is essential that the chamber remain free from air bubbles. The electrode is disengaged from the needle, plunged immediately into the bath and the connection made rapidly. The temperature of the bath will usually have fallen to 38° C. and can be checked with the thermometer and adjusted, if necessary, by adding warmer or colder KCl solution. A steady E. M. F. is obtainable within 30 to 40 seconds after the electrode is attached to the pH meter.

As soon as the reading is obtained the electrode is removed and the vessel washed free of blood by drawing in warm distilled water. It is then rinsed and refilled with oxalate in preparation for the duplicate determination. During the interval necessary for the pH reading and the washing of the vessel a syringe may be attached to the needle and a blood sample taken for analysis. This total procedure can be done in from 2 to 4 minutes. When stasis is avoided, duplicate determinations check within the error of the instrument ( $\pm 0.01$  pH). If this agreement is not obtained, a third determination should be done. Before each determination, we find it advisable to calibrate the electrode with standard buffers in the pH range of blood at 38° C., using the procedure as outlined for blood.

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<sup>1</sup> Both types of vessel were made for us by Mr. J. D. Graham, at the University of Pennsylvania.