Goodner of the Rockefeller Institute have shown in a small group of rabbits that the majority of the sera contained from one to two mg antibody nitrogen per cc, and one exceptional serum contained approximately 6.4 mg.

Similar experiments are being carried out with pneumococci of Type I. The results indicate that the method described above is applicable to production of Type I antipneumococcic serum.

Jules Freund MARTHA A. BEHAN DEPARTMENT OF HEALTH OF THE CITY OF NEW YORK BUREAU OF LABORATORIES. ANTITOXIN LABORATORY, OTISVILLE, N. Y.

## THE SIGNIFICANCE OF THE AMINO ACIDS IN CANINE NUTRITION1

In previous publications from this laboratory<sup>2,3</sup> it has been shown that only ten of the twenty-two amino acids known to exist in proteins are indispensable dietary components. These are tryptophane, lysine, histidine, phenylalanine, leucine, isoleucine, threonine, methionine, valine and arginine. With the exception of arginine, the removal of any one of these compounds from the food leads to a profound nutritive failure, accompanied by a rapid decline in weight, loss of appetite and eventual death. On the other hand, the exclusion of arginine from the ration is followed by much less pronounced effects. The subjects continue to gain, but at a suboptimal rate. This is accounted for by the fact that arginine, in contrast to the other members of the indispensable group, can be manufactured by the cells.<sup>4</sup> but at a speed which does not quite keep pace with the demands of normal growth. The twelve remaining amino acids are dispensable in the sense that they can be synthesized in adequate amounts out of. materials ordinarily available in the organism.

In arriving at the above conclusions young rats served as the experimental animals. Consequently, it does not follow necessarily that the findings are applicable to other species. Indeed, a quantitative difference in the arginine requirement has already been recorded in the literature. The growth of chicks is said to be accelerated by the addition of this amino acid to the ration, even when the latter contains 18 per cent. of casein<sup>5</sup> (equivalent to a dietary arginine con-

4 C. W. Scull and W. C. Rose, Jour. Biol. Chem., 89: 109, 1930.

tent of approximately 0.68 per cent.). This amount of casein furnishes more than three times the quantity of arginine necessary for the growing rat.<sup>2</sup> It becomes of importance, therefore, to ascertain whether the rat is singularly proficient in the synthesis of amino acids, or whether other mammals also manifest the ability to thrive on relatively simple mixtures.

The nutritive rôle of the individual amino acids has now been established for the adult dog. This was undertaken as a preliminary to extensive investigations on the maintenance of nitrogen equilibrium in various species by oral and intravenous alimentation. The program has advanced sufficiently to warrant a brief report at this time. The experimental findings and the details of the technique will be presented elsewhere.

Adult females were used throughout. As a rule, they were first brought into nitrogen equilibrium upon a casein diet. and were then transferred to a similar ration in which mixtures of highly purified amino acids served as the sole sources of nitrogen, except for traces introduced unavoidably as contaminants of the vitamin B concentrates. The urine samples were collected by catheterization at intervals of twenty-four hours, and the feces were divided into periods of seven days by the administration of carmine capsules.

The first dog received a ration containing the ten amino acids found previously to be indispensable for the growing rat. She promptly manifested a slight positive nitrogen balance, and continued to do so, with a moderate gain in weight, for the duration of the test (4 weeks). Obviously, the amino acids which are dispensable for the growing rat are also dispensable for the adult dog. At the beginning of the fifth week, arginine was dropped from the diet. The change was without influence upon either the body weight or the nitrogen balance of the subject. Thus, for the adult dog arginine is not a necessary dietary component. This finding was not unexpected. Nearly two years ago we<sup>3</sup> predicted that arginine would prove to be dispensable for full-grown animals. The experiment was discontinued at the end of the eighth week, at which time the dog was in perfect nutritive condition.

Three dogs were employed in determining the physiological importance of the other amino acids of the indispensable group. Invariably, the removal from the food of any one of these compounds was followed by a pronounced negative nitrogen balance. Furthermore, the restoration of the missing amino acid to the diet uniformly resulted in a positive nitrogen balance. These data demonstrate that the qualitative amino acid needs of the dog are identical with those of the rat. The fact that two widely different species require for their well-being the same components of the protein molecule, increases the probability that other mammals, including man, may manifest like responses.

In the experiments herein described the amino acid

<sup>&</sup>lt;sup>1</sup> The researches upon which this report is based were supported in large measure by a grant from the Rockefeller Foundation.

<sup>&</sup>lt;sup>2</sup> W. C. Rose, SCIENCE, 86: 298, 1937. <sup>3</sup> W. C. Rose, *Physiol. Rev.*, 18: 109, 1938.

<sup>5</sup> A. Arnold, O. L. Kline, C. A. Elvehjem and E. B. Hart, Jour. Biol. Chem., 116: 699, 1936; A. A. Klose, E. L. R. Stokstad and H. J. Almquist, Jour. Biol. Chem., 123: 691, 1938.

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 threenine 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.

UNIVERSITY OF ILLINOIS, URBANA

WM. C. ROSE ELDON E. RICE

## 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  $(1\frac{1}{2} \text{ miles})$ . 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  $8 \times 11$  inches at 2.5 feet and  $20.5 \times 26$  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.

E. NEWTON HARVEY

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

PRINCETON UNIVERSITY

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

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

<sup>&</sup>lt;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

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

<sup>&</sup>lt;sup>2</sup> Wm. Beebe, ''Half Mile Down,'' Harcourt, Brace and Company, New York, 1934.