Comments and Communications

Concerning a Dog's Word Comprehension

It is difficult to be sure of the extent that animals are able to generalize the meaning of those words to which they do definitely react. Data in this connection are as apt to come from spontaneous occurrences as from outright experiment.

To us the word *table* means something with a top, any kind of top, with four legs most frequently, any kind of legs, that is any size, any shape, and is used for a variety of purposes. Can a dog know *table* in that sense? Is a table anything but a special object with which the dog has special associations?

A woman who owns a large male English setter named Topper had given him a bone periodically. This always had occurred in the kitchen. Accompanying the act she always had said "Table, Topper," whereupon Topper had promptly carried the bone under the kitchen table. The woman, of course, had merely wanted to keep the rest of her kitchen floor clean. The table is a good-sized kitchen table.

Now one day there was a party in the living room. There were candy wrappers. Topper saw them and went to work on them. The woman immediately said: "Table Topper." She meant that he should take the papers into the kitchen under the table and chew them there. Instead, the dog seemed confused a moment, looked around, then deliberately walked to a small decorative coffee table, squeezed himself under it with difficulty, head far out in front, rump far out behind, and there confidently chewed. Plainly *table* in that dog's mind stood for something general, with at least two quite different examples. I invited the woman and her dog to my laboratory. I gave him a bone. At the woman's command he instantly went under a laboratory table. We tested him with all kinds of tables. The dog always went under the nearest table, never under the nearest chair or into a corner or a niche. Some weeks later the woman called me. She and the dog had been out in the large field back of their house, and the dog had dug up a buried bone. Almost idly, the woman had said: "Table, Topper." She had thought there was no table but she was wrong. At the end of the field, definitely out of sight, was an old picnic table that had been left there the previous summer. Topper knew about that table, and there he patiently carried the bone and munched.

To me it appears that the "Table, Topper" has become a stimulus for an action that includes the understanding of *table* as a fairly extended concept. In the last situation the dog has even laboriously searched out a table that he remembers and that the woman has forgotten. That is, this dog seems to understand the word with something approaching the spread of implications that it has for us.

GUSTAV ECKSTEIN

Department of Physiology,
University of Cincinnati

Interference with the Ultramicro Ascorbic Acid Method of Lowry, Lopez and Bessey

The ultramicro method of Lowry, *et al.* (*J. biol. Chem.*, 1945, **160**, 609) for the determination of ascorbic acid was used without special difficulty for nearly a year. Interference was subsequently encountered and since it may have been observed in other laboratories using this method, our experience with it and the means of avoiding it are herewith described. Interference was first encountered when an orange-red precipitate formed immediately after the addition of the dinitro-phenyl hydrazine reagent. This became progressively worse and occurred with blank, standard, and sample tubes alike. The precipitate dissolved only slightly upon addition of sulfuric acid and could not be completely removed by centrifugation. When it was possible to obtain a reading with the spectrophotometer on the reaction mixture, the values were high.

In an intensive effort to determine the cause of this precipitate, each of the reagents, including the distilled water, was systematically tested; the deep freeze storage unit was inspected for leaks of refrigerant; and other possible causative factors were sought. Finally, the cut-off ends of rubber sleeve stoppers, recommended by Lowry, *et al.* for sealing the micro tubes, were tested by extraction with 5% trichloroacetic acid, the protein precipitant used in the method. When this extract was added to the dinitro-phenyl hydrazine reagent, the characteristic precipitate formed immediately and consisted of the same needle-like crystals previously observed in the complete method.

A possible explanation for the failure of this interfering precipitate to develop when the method was first employed may be found in the fact that the rubber stoppers arrived with a protective coating. It was not entirely removed by the simple soap and water washing procedure. Perhaps the coating later wore off sufficiently to permit the liberation of some compound capable of reacting with the trichloroacetic acid and the dinitro-phenyl hydrazine reagent to produce the precipitate observed. Soaking the stoppers overnight in a 1N solution of sodium hydroxide in 80% alcohol, followed by thorough rinsing with distilled water, merely hastened the development of the precipitate.

The method is now successfully carried out by sealing each micro tube with a tiny oxygen-gas flame which closes it quickly without heating the tube contents or harming the ascorbic acid. Doubtless other satisfactory means of sealing these tubes can be found, but they should be tested first for possible interference with the reaction.

The chemical composition and properties of the precipitate are under investigation by one of us (R.R.S.)

at the Iowa State College Laboratories.

RUTH L. GOODLAND, ROBERT R. SEALOCK, NEVIN S. SCRIMSHAW, AND LELAND C. CLARK

University of Rochester School of Medicine and Dentistry, Rochester, New York; Iowa State College, Ames, Iowa; and *Fels Research Institute, Antioch College, Yellow Springs, Ohio*

Aerating Liquids by Agitating on a Mechanical Shaker

Kluyver and Perquin's *Schüttelkulturmethode (Biochem. Z.,* 1933, 266, 68) has become a popular method of studying the physiology of molds. This method involves continuous agitation of submerged cultures on shaking machines and assures a more uniform supply of nutrients and oxygen to all cells as well as a more uniform removal of gaseous waste products than does the surface culture method. During studies on the metabolism of *Penicillium chrysogenum,* generously supported by the Bristol Laboratories, it became desirable to determine how much greater the potential supply of oxygen is in shaken media than in media kept stationary. Since certain media foam quite vigorously when shaken, the question of how seriously such foams interfere with the diffusion of oxygen also needed consideration.

Experiments to measure the rate at which oxygen diffuses into media would be most significant from a physiological point of view when performed with media that contain respiring cells. Unfortunately such experiments are also the most laborious to set up. For example, one could suspend varying amounts of cells in shake-flasks and observe the rate at which they take up oxygen. A graph of "volume of oxygen absorbed per unit time" plotted against "amount of cells" would indicate a direct proportion between the two until a further increase in cells no longer gave an increase in respiration. This would mean, provided that the supply of oxidizable substrate is adequate, that the rate of respiration has become so high that it is limited by the rate of oxygen diffusion into the medium. The rate of oxygen uptake corresponding to the horizontal portion of the curve would then be a measure of the rate at which oxygen diffuses into the medium. (Cf. Umbreit, Burris, and Stauffer. *Manometric techniques.* Minneapolis: Burgess, 1945, p. 9.)

One could, of course, determine the rate of diffusion more simply in the absence of respiring cells. These determinations unfortunately are subject to the criticism that any conclusions which they may suggest are not necessarily applicable to living systems. However, since at least preliminary information can be obtained in this manner, such determinations were made as follows: 150 ml of freshly boiled and rapidly cooled distilled water, sometimes containing added substances, were placed in 500-ml Erlenmeyer flasks, which were then plugged with cotton, and agitated at 28° C on a reciprocating shaker, having a 4-in stroke and shaking at a rate of 85 strokes per min. At zero time and at intervals thereafter, the amount of oxygen dissolved was determined by the Winkler method (American Public Health Association, *Standard methods for the examination of water and sewage,* New York, 1946). All determinations were made at least in duplicate.

Fig. 1 gives some typical absorption curves. Oxygen dissolved in agitated liquids very rapidly, nearly saturating the liquid within a few minutes. Diffusion into still water proceeded slowly, as expected. In all cases the rate of absorption was most rapid at the beginning of the experiment, and slowed down as saturation was being reached. Assuming the ideal case in which oxygen is used by the organisms as fast at it is furnished, one can estimate the potential oxygen supply from these high initial rates. For example, under our conditions oxygen diffused into shaken distilled water during the first 30 sec at a rate of about 30 ml of oxygen per hr per 150 ml of distilled water, which would theoretically support the growth of 1 g (dry weight) of cells having

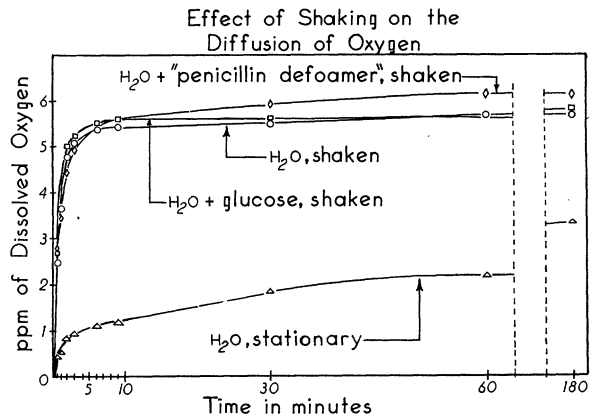

FIG. 1. The rate at which oxygen diffused during the first 30 sec, in ppm per hr per 150 ml of liquid was: 300 for shaken distilled water; 50 for stationary distilled water; 330 for shaken distilled water containing 0.05 ml penicillin defoamer; and 320 for distilled water containing 0.5% glucose.

a Q_{O_2} of about 30. This estimate is made with reservations, since the presence of high concentrations of nutrients and of cells will reduce the solubility of oxygen.

Not only did oxygen diffuse six times more rapidly into agitated water than into quiet water, but the total amount of oxygen present after 3 hr was 70% greater in the shaken than in the undisturbed water. Furthermore, it also seems likely that until the still water becomes completely saturated with oxygen there is an uneven distribution of oxygen; however, no determinations were made to test the validity of this assumption. The addition of 0.05 ml of Swift's "penicillin defoamer," an antifoam agent used in the manufacture of penicillin, did not appear to have a significant effect on the diffusion of oxygen; neither did the addition of 0.5% glucose.

It was at first difficult to find an artificial system that might be comparable to a medium covered by a foam, because the various foams tested in preliminary ex-