position is given in Table 1. The chicks were reared in an electrically heated battery, equipped with raised wire floors. The water was changed daily and the containers were cleaned by brush. At 4 wk of age, 1 ml of blood was drawn by heart puncture and the clotting time for whole blood was recorded. The results are shown in Table 2, column A.

Statistical analysis of the data showed that terramycin and arsonic acid prolonged the blood clotting time significantly at $P < 0.05$, whereas arsanilic acid was almost significant at the 0.05 level, as compared with the nonsupplemented diet. Vitamin K (Menadione), on the other hand, significantly reduced the blood clotting time.

To test the hypothesis that the action of these supplements on the clotting time was due to a lack of available vitamin K, a curative level of Menadione (20 mg/kg diet) was added to each ration and the clotting time was again determined after 72 hr. These results are shown in Table 2, column B. It appears that the addition of Menadione reduced the blood clotting time to normal in every case. The difference in the number of birds in columns A and B resulted from two causes: (*a*) two individuals from each lot were removed for histological studies, and (*b*) a number of birds died after the heart puncture. For the histological examination, birds with a relatively long clotting time were selected.

Although it is clear that certain of the supplements prolonged blood clotting, the exact mechanism remains to be determined. The supplements could affect the metabolism of bacteria synthesizing the vitamin, or exert their influence by altering number and/or type of bacteria in the intestinal tract. The possibility of these supplements functioning as antagonists is not excluded.

References

1. ALMQUIST, H. J., and STOKSTAD, E. L. R. *J. Nutrition*, **12**, 329 (1936).
2. ALMQUIST, H. J., PENTLER, C. F., and MECCHI, E. *Proc. Soc. Exptl. Biol. Med.*, **38**, 336 (1938).

Manuscript received June 29, 1953.

The Preparation of Latex Casts of Soil Cavities for the Study of Tunneling Activities of Animals

Murvel R. Garner[1]

Department of Biology, Earlham College, Richmond, Indiana

As part of a study of the roles of soil animals in relation to soil structure and fertility, it became necessary to obtain a clearer picture of the tunnels made by these animals. It was found that by pouring liquid latex into such openings as the tunnels of earthworms, ants, and spiders, and into shrinkage cracks in soil, casts could be made and preserved. These casts have revealed much about the tunneling and feeding habits that would have been difficult to discern otherwise.

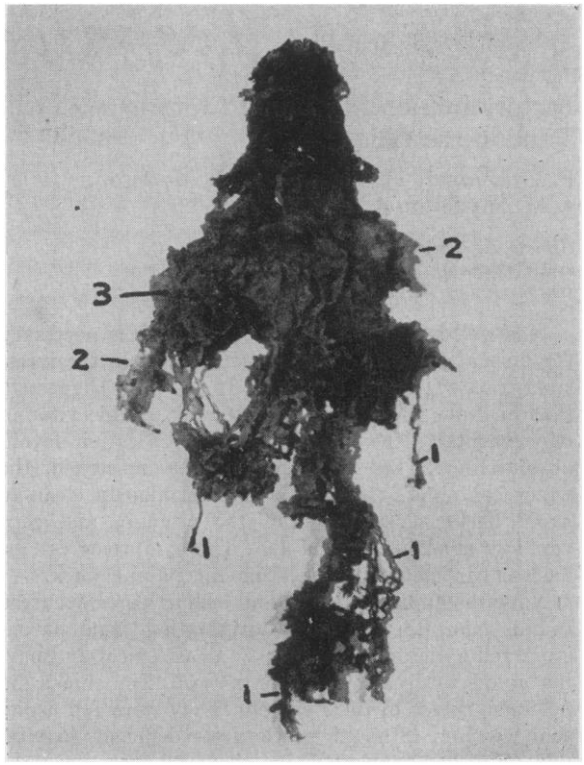

FIG. 1. Latex model of large interconnected soil voids, made by pouring latex into a surface opening in garden soil at Richmond, Indiana: (1) earthworm tunnels; (2) "drought cracks"; (3) walls roughened by "nibbling" of ants. About one-half natural size.

The simplest technique is carried out as follows. Liquid latex is thinned to a suitable consistency with commercial ammonia, diluted 1 to 8 with distilled water. The thinned material is poured into open holes until they are filled. The latex is allowed to harden for 2 or 3 days. The cast is then dug up and cleaned by washing under a stream of water.

The size of openings penetrated is controlled by the consistency of the latex. In some experiments the latex has been considerably diluted by ammonia water to which small amounts of the detergent and wetting agent (Joy) have been added. Also, some of the soil samples were partially evacuated in order to enhance penetration. For special purposes the latex may be colored with fat-soluble dyes.

The nature of the tunneling activities of animals has been revealed more effectively by this means. The casts show that in good garden soil near Richmond, Indiana, earthworms make a complex set of horizontal tunnels an inch or so beneath the surface of the ground. These connect by vertical shafts with other complex sets of horizontal tunnels in the decaying litter at the bottom of the furrow, and with other vertical shafts going 2 or 3 ft into C-horizon of the soil. The tunnels of earthworms are recognizable by the

[1] The writer is indebted to Elbert Ladd and the Naugatuck Chemical Company for a supply of latex, and to Charles F. Kettering and the Kettering Foundation for a grant to Earlham College for soil investigation.

glaze of mucus lining them. This glaze often effectively prevents the lateral seepage of the liquid latex and confines it to a single system of tunnels.

In contrast, the tunnels of ants have surfaces that are greatly "nibbled". It is apparent, also, that ants may utilize the upper part of an earthworm tunnel shaft as the entrance to their own nests. The mucus lining of earthworm tunnels seems to serve as a culture medium for fungi that attract ants. The upper portions of an earthworm tube become enlarged and roughened by the action of ants.

In addition to making possible the casts of the tunnels of subterranean animals, the latex method has been used to reveal the nature and extent of shrinkage cracks in dry seasons. It has shown in three dimensions the nature of continuous, interconnected voids in soil and the quality of the channels by which soil and water may move from one horizon to another.

It is believed that this technique offers further possibilities for the better understanding of the impacts of animals on soil formation and modification, and of the nature of the larger interconnected voids in the soil (Fig. 1).

Manuscript received June 10, 1953.

The Quantitative Determination of Adrenaline and Noradrenaline in Mixtures[1, 2]

Harold Persky and Sidney Roston

Institute for Psychosomatic and Psychiatric Research and Training, and *Department of Metabolic and Endocrine Research, Michael Reese Hospital, Chicago, Illinois*

A method for the determination of "total adrenaline-like substances" in blood has recently been reported (*1*). The method is based on the fluorimetric determination of the reaction product obtained by the condensation of the catechol amine with ethylenediamine (*2*). The 436-mμ line of a mercury arc was used for excitation and the total fluorescence above 500 mμ was measured. By this technique, the two principal sympathetic substances present in the body, adrenaline and noradrenaline, could not be differentiated in mixtures. Each substance, however, formed a separate condensation product with ethylenediamine, the adrenaline derivative fluorescing with five times the intensity of the noradrenaline analog.

The difference in fluorescence emission between these two compounds suggested that the fluorescence spectra of these substances might differ in shape. In order to test this hypothesis, the fluorescence spectrum of each condensation product was determined for 2 exciting frequencies: the 365-mμ and 436-mμ lines of the mercury arc. The apparatus employed was a model DU Beckman spectrophotometer modified to function as a fluorescence spectrophotometer in a fashion described elsewhere (*3*). The results are shown in Fig. 1.

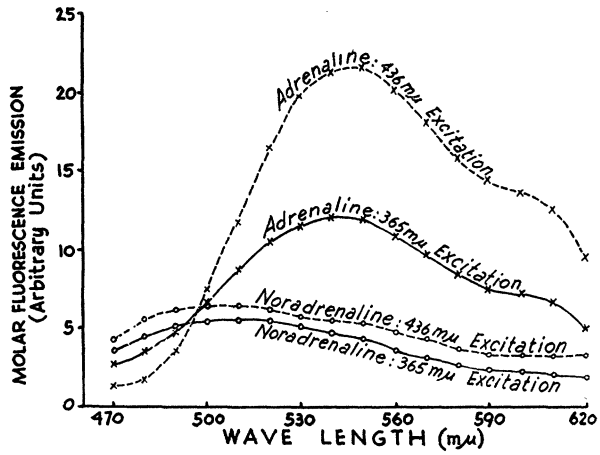

FIG. 1. The fluorescence spectra of the ethylenediamine condensation products of adrenaline and noradrenaline obtained with 2 exciting frequencies. The spectra were obtained with concentrations of 5.5×10^{-9} mole/ml and 21.9×10^{-9} mole/ml of adrenaline and noradrenaline, respectively.

It is seen that the adrenaline peak emission is further toward the red end of the spectrum than the noradrenaline peak by 30 mμ and 50 mμ for the 365-mμ and 436-mμ excitations, respectively. The intensity of fluorescence of the adrenaline derivative at its peak emission is 2.2 and 3.5 times greater than that of the noradrenaline for the 365-mμ and 436-mμ excitations. It is to be noted that the fluorescence emission of the adrenaline derivative on excitation with the 436-mμ line is almost twice that obtained with the 365-mμ line, whereas the noradrenaline analog shows no such increase with change of exciting sources. These differences in spectral characteristics provide a basis for the quantitative differentiation of these two important hormones in biological mixtures.

The technical aspects of the analytical differentiation were simplified by employing a Farrand photoelectric fluorometer. The primary consisted of Corning filters No. 5113 and 3389 which pass the 436-mμ mercury arc line. Two different secondaries were employed, the first consisting of Corning filters No. 5433 and 3384 which pass a narrow band peaking in the region of 510 mμ and the second consisting of Corning filter No. 2418 which cuts off below 600 mμ. These particular filters were employed because they yielded maximum differentiation between the adrenaline and noradrenaline derivatives and minimum blanks. The fluorescence of the mixture was recorded with each secondary filter combination and 2 simultaneous linear equations were solved for the content of adrenaline and noradrenaline in the mixture. The coefficients of the equations were determined from separate measurements of standard solutions of adrenaline and noradrenaline in the same fashion.

[1] This work was supported in part by the Flora Warner Lasker Memorial Research Fund.

[2] We wish to thank Oliver H. Lowry of the Department of Pharmacology, Washington University School of Medicine for allowing us to use his fluorescence spectrophotometer and for advice and assistance in this study.