studied the chemical and physical properties of this venom and have found that it does not resemble the venom of any stinging insect previously studied.

Venom was collected from major workers taken in the field during the fall and winter. The ants were held by the petiole with a forceps while the tip of the abdomen was stroked with a fine capillary until the sting was everted. Droplets of venom issuing from the tip of the sting were collected in the capillary. The procedure was carried out conveniently under low magnification with a dissecting microscope.

The venom is water-insoluble, being less dense than water, in which it disperses as fine milky-colored globules. The absence of ninhydrin-positive reactants indicates it is nonproteolytic. The venom consists of two phases, primarily being composed of an alkaline carrier which suspends fine droplets of a greater density. The alkalinity of the mixture is not due to metal ions. These were determined to be absent by emission spectrographic examination in the Jarrell-Ash 4.8-meter grating spectrograph. The venom is soluble in most organic solvents, but least soluble in ethanol.

Ultraviolet spectrophotometric examination of the venom (in ethanol) in a Beckman DU spectrophotometer showed no peaks, absorption being strongest at the lower wavelengths. Infrared examinations (3) were made on a Perkin-Elmer model 21 spectrograph either as a carbon tetrachloride solution or as a film of venom applied directly to the rock salt prism. Only aliphatic C—H stretching was found (3.4 μ), demonstrating the nonaromatic nature of the venom. A carbonyl group (5.70 μ) is present which does not appear to be an open chain, simple ketone (4). Both methyl (7.25 μ) and methylene groups are present as well as a possible ether linkage (8.6 μ). The C—H/C=O ratio was found to be much higher when the sample contained small amounts of suspended globules. This indicates that the globular component contributes most or all of the carbonyl-containing compound.

Insecticidal activity was examined by exposing insects to residues, or by topically applying the venom as obtained from the ants. Samples for residual determinations were prepared as acetone or ethanol solutions. The venom was found to be highly toxic to the fruitfly, *Drosophila melanogaster* Meig., the housefly, *Musca domestica* L., a termite, *Kaleotermes* sp., the boll weevil, *Anthonomus grandis* Boh., and the rice weevil, *Sitophilus oryza* (L.). In addition, two species of mites, *Tetranychus telarius* L. and *T. cinnabarinus* Boisd., were highly susceptible. Interestingly, the fire ant is not highly susceptible to its own venom.

The antibiotic activity of the venom

was investigated, and it was shown that several types of microorganisms were inhibited by a 1/50 dilution. Tests made by the paper-disk method demonstrated the effectiveness of this venom against *Micrococcus pyogenes, Streptococcus pyogenes, Escherichia coli, Lactobacillus casei,* and a variety of molds. The antibiotic activity of fire ant venom probably explains why the pustules arising at the site of the sting are antiseptic (2). A thorough study of the antibiotic properties is now being made.

The toxicities of different samples of venom to *Drosophila* have been found to vary, some samples being at least as toxic as DDT. Highly toxic samples of venom produce an instantaneous paralysis highly suggestive of a nerve poison. The most toxic samples contain a large percentage of the globular component, which suggests that this phase represents the toxic principle.

Recent work on the chemistry of ants has demonstrated the presence of a terpenoid lactone, iridomyrmecin (5), in various species of ants in the subfamily Dolichoderinae. Although these ants are in a phylogenetically more advanced subfamily than the fire ant (Myrmecinae) and do not have a functional sting, our infrared data suggest similarities in structure to this lactone. Iridomyrmecin also has been shown to have antibiotic and insecticidal activities (6). However, whereas iridomyrmecin produces tremors in insects suggestive of DDT poisoning (7), fire ant venom produces a sedative reaction, paralysis being unaccompanied by tremors.

The chemical composition of fire ant venom and the effect of it on malignant cells are being studied.

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14 March 1958

Methionine Inadequacy of Casein Hydrolyzate as Source of Difficulty in Vitamin Assays

The microbiological estimation of vitamins is undertaken routinely in many laboratories. Despite all efforts to maintain assay procedures under rigid control, it is not uncommon to encounter difficulty suddenly with methods that had been proceeding smoothly. Such occurrences are sometimes attributable to deterioration of one of the solutions used in making the medium, or, less frequently, to mutation of the test organism. In other instances, despite much searching, no reason for the difficulty can be found—then, suddenly, one once again obtains a satisfactory standard curve. Recent experience in this laboratory leads us to suggest that an unsuspected source of difficulty may be the variability in amino acid content of batches of commercial vitamin-free casein hydrolyzate (acid).

The routine analysis of folic acid by the AOAC method (1) with *Streptococcus faecalis* 29-21 [isolated by Harrison (2)] as the test organism suddenly failed, as evidenced by a very flat standard curve. Although the *assay* medium no longer supported the usual level of growth of *S. faecalis,* the organism still grew well on *nonsynthetic* inoculum broth. Doubling the concentration of certain batches of casein hydrolyzate in the assay medium resulted in improved growth, but the degree of improvement varied greatly from batch to batch.

The effect of supplementation of the folic acid assay medium with the following amino acids was studied: L-arginine · HCl, L-asparagine, L-cysteine · HCl, L-cystine, L-glutamic acid, L-histidine · HCl, DL-isoleucine, L-leucine, DL-lysine, DL-methionine, DL-serine, DL-threonine, DL-tryptophan, and DL-valine. For each amino acid, the amount added was that indicated by Greenhut et al. (3) to be necessary for the optimal growth of *S. faecalis,* American Type Culture Collection No. 8043. Only two of these amino acids produced any significant effect: the supplement of DL-methionine (5 mg/100 ml of double-strength medium) permitted normal growth of *S. faecalis,* while the supplement of L-leucine slightly improved total growth. The effect of leucine later proved to be due to contamination of this amino acid with 9 percent methionine.

The various batches of casein hydrolyzate were analyzed for methionine by the method of McCarthy and Sullivan (4), and the concentration of four lots on hand was found to be 0.15, 0.24, 0.30, and 0.38 mg of L-methionine per milliliter of hydrolyzate (10 percent casein). When the hydrolyzates were assayed by the microbiological method of Steele
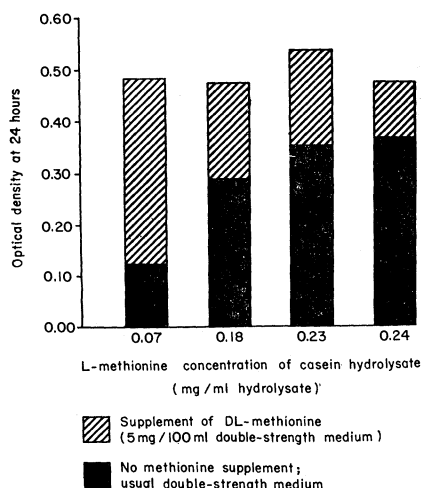
Fig. 1. Effect of methionine supplementation on total growth of *Streptococcus faecalis* 29-21 in folic acid assay medium. (Total growth occurring under conditions represented by 5 ml of double-strength medium and 10 mµg of folic acid, in a total volume of 10 ml.)

et al. (5), however, the corresponding values were 0.07, 0.18, 0.24, and 0.23 mg of L-methionine per milliliter of hydrolyzate. The latter data were considered to reflect more nearly the true methionine concentrations than the former, when it was noted that off-colors sometimes resulted in the colorimetric procedure, particularly when certain samples were assayed at high concentrations. A direct relationship existed between methionine concentration of the hydrolyzates and their varying abilities to support the growth of *S. faecalis* (Fig. 1), but even the batch of hydrolyzate with the greatest methionine concentration did not contain enough to permit optimal growth of the organism.

In addition to the strain of *S. faecalis* used in this laboratory, a culture of *S. faecalis* 8043, the strain most commonly employed for folic acid analyses, was also studied. Similar results were obtained with this organism as with *S. faecalis* 29-21—that is, supplements of methionine resulted in improved growth, but the effect of methionine was not as marked as with *S. faecalis* 29-21, for the latter requires about 30 percent more methionine for maximal growth at 24 hours than does *S. faecalis* 8043. Even to meet the requirements of *S. faecalis* 8043 for optimal growth, however, Greenhut et al. (3) suggest a concentration of 0.25 mg of DL-methionine per assay tube containing 10 ml of single-strength medium. This is, in reality, a requirement of 0.125 mg of L-methionine per tube, since the D-isomer is utilized only slightly, if at all (6). With the usual concentration of casein hydrolyzate of 0.5 ml/10 ml single-strength medium, the hydrolyzate must contain 0.25 mg of L-methionine per milliliter of hydroly-

zate to provide the requisite amount, yet only two of the four hydrolyzates tested approached this amount. It seems rather noteworthy that the reported (7) methionine content of casein of 3.3 g/16 g of nitrogen (equivalent to approximately 3 mg/ml of a 10 percent casein hydrolyzate) exceeds by more than ten times the amount actually found in these hydrolyzates, implying a rather extensive and somewhat variable loss of methionine during hydrolysis. To allow for this, it is now routine in this laboratory to supplement all folic acid assay medium with 5 mg of DL-methionine per 100 ml of double-strength medium.

It has long been recognized that methionine is an amino acid required by many common assay organisms. Thus, a deficiency of this amino acid would adversely affect a number of assays utilizing a variety of test organisms. Correspondence with the manufacturer of the hydrolyzate revealed that no recent changes had been made in the manufacturing process and that the variability observed might be encountered under ordinary manufacturing conditions. The possible inadequacy of the usual amounts of casein hydrolyzate in meeting the amino acid requirements of various organisms should, therefore, be considered as a source of difficulty with microbiological assays.

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23 June 1958

"Root Pressure" in Gymnosperms

Movement of water to the tops of trees 200 or more feet high, in the large quantities which are required for normal growth and to replace losses by transpiration, involves the expenditure of enormous amounts of energy. There are two main theories current today of how this work is accomplished.

The one most widely accepted, the "suction tension theory" of Dixon and Joly (1) and of Askenazy (2) places the

energy expenditure at the surface of the leaf mesophyll cells in the form of heat of vaporization of water; vaporization sets up menisci in the porosities of the cell walls which in turn exert a tension against the water reservoir in the plant; this reservoir is pictured as being continuous through stem and roots with the water of the soil, and held against collapse by adhesion to the rigid framework of the plant's structure and by cohesion within the column. Water is thus *pulled* through the plant by the menisci at the leaf surface. The mechanical processes and structures involved require no active participation of the living protoplasts. Any accident which would break the column would destroy the effectiveness of the system.

Such a system can function only under conditions of (i) active transpiration, (ii) complete freedom from dissolved gases capable of causing cavitation and (iii) complete rigidity (freedom from shocks capable of breaking the adhesion of fluid to wall). Although this theory has a prominent place in present-day textbooks, its inadequacies have been pointed out repeatedly, most recently and forcefully by Scholander (3). Greenidge has also reviewed the subject (4).

The second theory postulates that energy is expended within the plant, probably in the root tips, endodermis and/or the medullary rays, against pressure gradients, comparable to the water-secreting mechanism of the mammalian kidney tubule which drives water back into the blood after its passive filtration in the glomeruli. Energy for this work would come from respiratory processes and would be independent of the physical phenomenon of transpiration, though affected by temperature, soil moisture, salt levels, carbohydrate availability, and other factors. Its immediate expression is the guttation which occurs from leaves on wet mornings and in the tropics where transpiration is reduced or lacking, and in the well-known exudation from cut stems. It was originally described by Hales (5) in 1727 and is commonly designated "root pressure."

Both mechanisms doubtless do operate, each under special conditions. Their relative importance in the water economy of plants is, however, still a subject of debate. Arguments against the importance of root pressure as a factor in sap movement have in general been three.

1) The observed pressures are generally too small to account for movements of water to heights of more than 30 or 40 feet. This argument was seriously weakened by the demonstration by White (6) in 1937 of secretion pressures exceeding 6 atm (about 200 feet) in single isolated tomato roots.

2) The amounts of water moved are