TABLE I.
Survival Times and Weight Changes of Rats Receiving High Dosages of Acetate.

Substance fed	(Daily dosage millimols per kg), No. of days survival	% metabolic rate due to acetate combustion
Acetic Acid	(30) 14, 14, 14; (40) 2, 2, 3, 3, 3, 3, 4, 4, 5, 5, 14, 14; (50) 2, 2, 3, 3, 3, 3, 4, 5, 5, 5, 6, 6; (60) 2, 2, 2, 2	4.2 to 8.4
Sodium Acetate	(30) 4, 14, 14; (40) 8, 13, 14; (50) 14, 14, 14; (60) 14, 14, 14; (70) 14, 14, 14; (80) 2, 14, 14; (90) 1, 3, 7, 14, 14; (100) 1, 2, 2, 14; (110) 1	4.2 to 15.4
Ammonium Acetate	(30) 14, 14, 14; (40) 8, 14, 14; (50) 4, 14, 14; (60) 1, 14, 14; (70) 1, 1, 4	4.2 to 9.7
$NaHCO_3$	(100) 14, 14, 14; (150) 1, 1, 1	
Water	14 (volume equal to largest dosage)	

Barnes, Carne and Wick[5] have reported gastrointestinal irritation with acetic acid and have used preferably sodium acetate. A difficulty in the use of the sodium salt is that excess sodium remains after combustion of the acetate radical and alkalosis develops. Alkalosis, however, is not the toxic factor since a dosage of 100 millimols per kg-day of NaOAc produced death in 1 to 2 days of 3 of a group of 4 rats while 100 millimols per kg-day of $NaHCO_3$ produced no fatalities in a group of 3 rats in 14 days. Alkalosis due to the sodium salt can be prevented by using the free acid or the ammonium salt. These two acetates while not producing alkalosis are both irritant to the alimentary tract and probably produce their toxic symptoms by injury to this organ.

When sodium acetate is given in 2 daily doses 70-80 millimols per day can be tolerated for 14 days. The total daily tolerable dose exceeds the single toxic dose of 55 millimols as found by Woodward, Lange, Nelson and Calvery.[7]

The main toxic symptoms observed just before death were loss of weight, blistering of the paws, and reddish appearance of nose and whiskers.

13819

Isolation of *Staphylococcus albus* from Hemolymph of the Roach, *Blatta orientalis.*

OSCAR E. TAUBER AND JAMES T. GRIFFITHS, JR.

Insect Physiology Laboratory, Department of Zoology and Entomology, Iowa State College, Ames, Iowa.

Previous examinations of hemolymph from the oriental roach, *Blatta orientalis,* have demonstrated that bacterial infections of the body fluid are associated with high total hemocyte counts[1] and high percentages of mitotically dividing hemolymph cells.[2] Since it is known that some insect leucocytes are phagocytic, the above responses may be interpreted as a mechanism whereby the numbers of hemocytes are increased for defense purposes. Thus a larger number of ingesting phagocytes would be available for combatting the invading organism. In addition, other symptoms indicated that presence of

1 Tauber, Oscar E., and Yeager, J. F., *Ann. Ent. Soc. Am.,* 1935, **28**, 229.

2 Tauber, Oscar E., *Ann. Ent. Soc. Am.,* 1940, **33**, 113.

the bacteria was decidedly harmful. Although occurrence of bacteria in insects has been noted many times,[3] descriptions often have been inadequate and identification impossible. Therefore, in this case, samples of infected hemolymph were carefully taken from a cut antenna, and transferred to various bacteriological media. Later, single organisms were isolated and cultivated in pure culture. The following characteristics were observed after 24 to 48 hr incubation:

Morphology: Spheres, 0.55 to 0.75 μ occurring singly or in irregular groups. Gram positive.

Agar colonies: Raised, circular, smooth, porcelain-white, glistening, entire.

Agar slant: Moderate growth, filiform, smooth, glistening.

Blood agar: β-hemolysis.

Broth: Turbid, with rather coarse, granular sediment.

Gelatin stab: Slow, saccate liquefaction.

Litmus milk: Slightly acid, with tendency to coagulate.

Potato: Thick, smooth, white glistening growth. Brown with age.

Indol: Not produced.

Nitrates: Reduced to nitrites.

Hydrogen sulphide: Produced.

Carbohydrates and alcohol fermented: Acid in dextrose, lactose, sucrose, and mannitol; negative in inulin and raffinose.

Oxygen relations: Aerobic and facultative.

Optimum temperature: 30°C.

When the above characteristics were checked in Bergey's Manual,[4] the isolated causative agent was found to compare closely to *Staphylococcus albus.** The lower optimum temperature (Bergey gives 37°C) may indicate the development of a strain of *albus* which has become accustomed to growth in a poikilothermic animal, rather than in or on a homeothermic form.

Pathogenicity of the described organism for the oriental roach was shown by various symptoms. Food was taken in small quantities, if at all, especially when the infection was severe. Slow and feeble movements pointed to a general body weakness. If placed on its dorsal surface, an afflicted specimen was often unable to right itself. Righting movements were uncoördinated and ineffective. Respiratory movements were irregular. Evidence of a neurotoxin manifested itself in a progressive anteriorly-moving paralysis. Metathoracic legs were affected first. If the animal was stimulated to move, these legs were useless and were dragged along. As the mesa- and prothoracic pairs became useless, spasmodic twitchings of the whole body also appeared. In the final stages of the disease, the legs were folded under the body; the head was tucked beneath the forelegs; and the whole animal became arched. This posture was maintained until death.

In addition to these external signs, and the increase in hemocyte mitosis, the circulating cells became filled with vacuoles. Many hemocytes underwent cytolysis. During the final stages of the affliction, affected hemolymph became thick and milky from the presence of tremendous numbers of parasitic bacteria.

Just how the organism made its initial entry into the roaches is not known. Infections were already established when the insects were trapped. However, transfer of the causative agent was easily accomplished by needle inoculation of normal roaches with diseased hemolymph, or with a broth culture of the isolated causative agent. After the disease became established, recovery of the organism was always possible. In all cases of successful inoculation, the roaches died from the effects of the affliction.

The incidence of this infection among roaches of this species is not known. All original infected specimens came in a shipment from a collector in Mississippi. Two other groups, totaling more than 500 individuals, from other localities, gave no evidence of the presence of the organism.

---

3 Paillot, A., *L'infection chez les insectes. Immunité et symbiose*, Patissier, Paris, 1933.

4 Bergey, D. H., Breed, R. D., Murray, E. D., and Hitchens, A. P., *Bergey's Manual of Determinative Bacteriology*, Williams & Wilkins, Baltimore, 1939.

* Identification of the organism was made by Dr. J. R. Porter, Department of Bacteriology, College of Medicine, State University of Iowa, Iowa City.

Summary. A bacterium, pathogenic for the roach, *Blatta orientalis,* was isolated in pure culture from the hemolymph of this insect, and proved to be *Staphylococcus albus,* as described in Bergey's Manual. Infections could be established in normal roaches by inoculation with diseased hemolymph or with broth cultures. Death was preceded by a characteristic progressive paralysis.

13820

Protective Action of Sulfanilamide Against Liver Cirrhosis from Chronic Poisoning with Carbon Tetrachloride.*

J. C. FORBES, B. E. LEACH AND G. ZUR WILLIAMS.

From the Departments of Biochemistry and Pathology, Medical College of Virginia, Richmond.

In a previous article[1] it was reported that oral administration of sulfanilamide, sulfathiazole or sulfapyridine prior to exposure reduced markedly the mortality of rats following acute poisoning by inhalation of carbon tetrachloride. Additional work on this problem dealing with the effect of sulfanilamide against chronic poisoning from carbon tetrachloride, as well as results obtained by use of other possible protective substances, is presented at this time.

These chronic poisoning experiments confirmed our previous observations. In preliminary experiments in which sulfanilamide was incorporated in the diet, (100 mg per 100 g of food) it was found that the animals ate well when no carbon tetrachloride was administered, but as soon as the periods of anesthesia were begun they practically refused to eat and consequently became more susceptible than the controls. This necessitated the administration of the drug by stomach tube in all subsequent experiments. One cm^3 of solution per 100 g of body weight was always used, the drug being either completely dissolved or with undissolved material in fine suspension in the water. Approximately 3 hr were allowed to elapse from the time of sulfanilamide administration before each period of anesthesia was begun. A summary of the experimental results is presented in Table I. It will be seen that there is a certain amount of overlapping but the general protective tendency is evident. In experiments 4 and 5, records of the daily food consumption were made. During the first few weeks no significant difference was noted between control and treated animals, but as the experiment progressed the food consumption of those receiving sulfanilamide was definitely greater than that of control animals.

The possible protective action of a variety of other substances has been studied in a number of acute poisoning experiments, but no protective action was obtained in any case. These substances included the following vitamin preparations, thiamine hydrochloride, riboflavin, pyridoxin hydrochloride, calcium pantothenate, nicotinic acid, inositol, para-aminobenzoic acid and ascorbic acid, and the following amino acids, *dl*-alanine, glycine, *l*-tyrosine and *dl*-methionine. Since para-aminobenzoic acid is known to inhibit the action of sulfanilamide against microorganisms, acute poisoning experiments were carried out in which this vitamin was administered simultaneously with sulfanilamide. No evidence of inhibition of the protective action of the sulfanilamide was obtained.

Discussion. The mechanism for the protective action of the sulfonamide drugs against carbon tetrachloride is obscure. Mackenzie and Mackenzie[2] have recently reported that

* Acknowledgment is gratefully made to the John and Mary R. Markle Foundation for its financial aid.

1 Leach, B. E., and Forbes, J. C., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 361.

2 Mackenzie, J. B., and Mackenzie, C. G., *Proc. Fed. Am. Soc. f. Exp. Biol.,* 1942, **1**, 122.