Ethylene Dibromide for Destroying Fruit Fly Infestations in Fruits and Vegetables

J. W. Balock

Bureau of Entomology and Plant Quarantine, USDA, ARA, Washington, D. C.

Three species of fruit flies[1] are established in Hawaii and constitute a serious threat to mainland agriculture. Losses caused by these flies to Hawaiian agriculture consist both in direct damage to crops by larval feeding and in the restrictions imposed by quarantines on the free movement of crops to mainland markets.

In the event of even an incipient infestation on the mainland by one or more of these flies, quarantine restrictions would probably involve the greater losses, unless satisfactory commodity treatments for eliminating fly infestations were available for the large quantities of fresh fruit and vegetables that might be grown in the infested area.

Vapor-heat and methyl bromide treatments have been developed and used successfully in the export from Hawaii of papaya, pineapple, tomato, bell pepper, and zucchini. However, recurrent injury has been reported occasionally even where the treatment has been carefully controlled. The cause of such injury is not clear. It has been variously attributed to season, rainfall, location, soil, varietal differences, and cultural and agronomic practices. Many quarantined commodities, such as bananas, avocados, string beans, and cucumbers, would not tolerate either treatment.

In the course of screening various materials as fumigants on naked eggs and larvae of the oriental fruit fly, it was found that ethylene dibromide was the most toxic of 53 compounds tested.[2] The LD_{95} concentrations at 70° F were 0.43 mg/l for eggs and 0.95 mg for third-instar larvae. Concentrations of methyl bromide required to give the same mortality were 24.5 and 18.5 mg/l.

In large-scale tests ethylene dibromide was used successfully as a fumigant to destroy the immature stages of the oriental fruit fly in papaya and guava and the melon fly in cucumbers and tomatoes. Complete mortality was obtained at dosages of ½ lb/1,000 cu ft for 2 hr at 70° F for the oriental fruit fly and at ¼ lb for the melon fly. In these studies 11,459 fruits with fruit fly infestations of 137,077 eggs and larvae were used. The liquid fumigant was volatilized by heating. Phytotoxicity tests with papaya, pineapple, avocado, Cavendish banana, bell pepper, zucchini, cucumber, and string beans showed no injury from the gas concentrations required to produce complete fruit fly mortality. A comparison of mortality curves shows that ethylene dibromide is approximately 17 times as effective as methyl bromide in destroying the immature stages of the oriental fruit fly in papaya.

Preliminary data indicate that ethylene dibromide shows the same order of toxicity to the Mediterranean fruit fly and the oriental fruit fly.

[1] The Mediterranean fruit fly, *Ceratitis capitata* (Wied.), the melon fly, *Dacus cucurbitae* Coq., and the oriental fruit fly, *Dacus dorsalis* Hendel.

[2] These screening tests were made by the author and D. L. Lindgren, University of California Citrus Experiment Station, Riverside, Calif.

Formation of a Competitive Antagonist of Vitamin B_{12} by Oxidation

J. M. Beiler, J. N. Moss, and Gustav J. Martin

Research Laboratories, The National Drug Company, Philadelphia, Pennsylvania

In the course of an investigation of the effect of various chemical procedures on vitamin B_{12} it was found that treatment of the vitamin in strong acid solution with hydrogen peroxide caused a decolorization of the solution. Tests of this solution showed that the reaction product exhibited a competitive antagonism to vitamin B_{12}.

To 10 ml of a B_{12} solution containing 100γ/ml was added 5 ml of concentrated HCl. A few drops of a 30% solution of hydrogen peroxide were added, with stirring at room temperature. The solution decolorized. It was allowed to stand for about an hour at room temperature and was then neutralized with NaOH. The activity of this solution was then assayed directly on *Lactobacillus leichmanii* 4797, using the method of Skeggs *et al.* (*1*).

The results, presented in Table 1, showed an inhibitory effect of the substance on the microorganism, which could be counteracted by vitamin B_{12}. Only at the highest level of inhibitor tested was there incomplete counteraction.

TABLE 1

EFFECT OF A B_{12} OXIDATION PRODUCT ON *Lactobacillus leichmanii*

Vitamin B_{12} (γ/tube)	B_{12} oxidation product (γ/tube)				
	0	5	10	25	50
0	0	0	0	0	0
0.001	210	175	140	25	0
0.005	270	200	220	137	0
0.05	300	310	282	230	0
0.5	286	295	275	240	0
5.0	298	310	305	280	25

Figures representing the concentration of the B_{12} reaction product are based on the original concentration of B_{12} in the starting material. The figures for bacterial growth are direct readings on the Klett-Summerson colorimeter, which was set at zero with the organism control.

The solution was also tested on *Staph. aureus, S. typhosa,* and *Ps. aeruginosa,* three organisms that do not require preformed vitamin B_{12} as a growth fac-

tor. No inhibitory effect was observed in these systems.

The substance formed in the reaction described above would thus, on the basis of these tests, appear to have a specific antagonistic effect to vitamin B_{12}, since it is counteracted by the vitamin and has no inhibitory effect where the vitamin is not an essential factor. The chemical structure is not known, although the fact that the solution was decolorized during the reaction would indicate that the cyanide-cobalt complex was attacked. That this complex could be broken up by permanganate oxidation was reported by Brink *et al.* (*2*). These authors, however, identified hydrocyanic acid as a reaction product and considered that they had converted vitamin B_{12} into vitamin B_{12a}. This is apparently not the case with the peroxide oxidation reported here, since free cyanide could not be found with the $FeSO_4$ test (*3*), and the reaction product has an antivitamin rather than a vitamin activity.

Further chemical and biological work on this material is in progress.

References

1. Skeggs, H. R., *et al. J. Biol. Chem.*, **176**, 1459 (1948).
2. Brink, M. G., Krehl, F. A., Jr., and Folkers, K. *Science*, **112**, 354 (1950).
3. *U. S. Pharmacopeia*, **XIV**, 721 (1950).

Functional Activity of the Sweat Glands in the Hairy Skin of the Dog

Tsuyoshi Aoki and Masao Wada

Physiological Laboratory,
Tohoku University School of Medicine, Sendai, Japan

The presence of the sweat glands in the dog not only in the foot pads but also over the body surface covered with hairs was described first by Gurlt in 1835 (*1*). His findings were confirmed by a number of investigators, and the literature was fully reviewed by Claushen (*2*). Nevertheless, we have found in several textbooks, monographs of physiology, and scientific encyclopedias a misleading description that in the dog sweat glands are found only in the foot pads. On the other hand, the lack of convincing evidence concerning the functional activity of the sweat glands in the hairy skin of the dog has hitherto led us to believe that this animal does not sweat over the general body surface.

The present paper is concerned with a demonstration of the sweating response in the hairy skin of the dog to some sudorific drugs and to local heating of the skin. For visualization of sweat we have used the iodine-starch method of Wada and Takagaki (*3, 4*), which proved to be suitable for this purpose. More than 30 dogs, mongrels and fox terriers, between the ages of 1 and 8 years were studied. Unanesthetized dogs were fastened to animal boards in either the supine or the prone position. The hairs of the regions to be tested were cut as short as possible, and the skin was painted first with iodine-alcohol solution and then with a starch–castor oil mixture. The sweating was designated by the appearance of black spots at each orifice of the hair follicle (Fig. 1). In the skin with black hairs, it was somewhat difficult to find the sweat spots when there was little sweating. The front aspect of thorax and abdomen and ventral surface of the thigh were chosen as the most suitable regions for observation. In most of the animals no spontaneous sweating was observed on the hairy skin during the whole time of the experiment, even during violent struggling.

The first tests of the functional activity of the sweat glands were made with intradermal injections of pilocarpine, acetylcholine, or adrenalin. Pilocarpine hydrochloride (JSP), acetylcholine (Roche), and adrenalin hydrochloride (Sankyo Co.) solutions were diluted with 0.9% NaCl to appropriate concentrations. One tenth or 0.2 ml of each solution was injected intradermally. With concentrations of $1:10^3$ to $1:10^5$ each of these three drugs was effective in producing visible sweating around the site of injection. The sweating by adrenalin was not inhibited by atropine, unlike the sweating by pilocarpine or acetylcholine.

The excitability of the sweat glands was measured by determining the minimal effective concentration of adrenalin for sweating, as tried previously with human sweat glands (*3*); it was found to be of almost the same order as that of the sweat glands in the trunk and extremities of healthy young men and women. The minimal effective concentrations of adrenalin ranged from $1:10^6$ to $1:10^8$, and those of acetylcholine from $1:10^8$ to $1:10^{10}$.

Another evidence of the functional activity of the sweat glands in the hairy regions was the fact that the sweating response was easily elicited by a local application of heat. The upper or lower portion of the trunk was introduced into a wooden cabinet ($50 \times 80 \times 60$ cm) and subjected to the radiant heat supplied by four 100-w electric bulbs. The response was observed through glass windows in the top and in the side walls of the cabinet. When the temperature inside the heating cabinet, which was measured at some distance above the skin surface, reached 30°–35° C, sweating was found to have been induced. In some dogs it occurred at a temperature below 30° C. Usually sweating was localized in the heated regions, and areas of skin outside the cabinet showed for the most part no sweating, in spite of the fact that the heat was so excessive as to cause severe panting and a considerable rise in rectal temperature. In a few dogs, however, spontaneous sweating was observed to occur slightly in some restricted areas—e.g., around the umbilicus and in the median part of the hypogastric region—when the upper portion of the body was intensively heated.

The local sweating induced by application of radiant heat was through the peripheral mechanism, since it was produced even in skin areas in which the nerve supply had been removed by sympathectomy either