In the present investigation it is extremely doubtful whether this new principle that influences chemically induced sarcomata is transmitted through the mother's milk, since there is ample evidence that foster nursing does not modify susceptibility or influence the rate at which the induced tumor appears following the injection of the carcinogen (unpublished data of author).

The present investigation is of significance, since it demonstrates that a new principle, presumably of a biochemical nature, has influenced the production of a malignant tumor. This agent varies in amount or potency with advancing age and is transmitted to the next generation. Perhaps when it is identified, other phases of the cancer problem, such as prevention and spontaneous regression, may be elucidated. Susceptibility to cancer is, of course, only the obverse of resistance to cancer. The present evidence indicates equally clearly the existence of a new *resistant* mechanism that is capable of changing the rate at which an offspring of a given female develops sarcoma in the presence of methylcholanthrene, that it is highest in young breeding females and diminishes in effectiveness with advancing age. It is in the young animal that this resistant mechanism for the control of some characteristics of cancer must be sought.

By the use of F_1 individuals that will grow the normal tissues of both ancestral stocks, the elucidation of this mechanism for resistance to cancer in young animals will be considerably aided.

Antibacterial Action of the Blood of the Large Milkweed Bug[1]

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The blood of the large milkweed bug, *Oncopeltus fasciatus,* contains an antibacterial agent active *in vitro* against *Staphylococcus aureus* and one strain of *Bacillus subtilis.* The active principle is water soluble, stable to boiling for 30 min but destroyed by autoclaving, and active at a dilution of at least 1 part in 10,000.

The large milkweed bug, a well-known laboratory insect, may be kept in culture throughout the year, with little care and expense, feeding on milkweed seeds and water (*7*). It is prolific and requires only about 3 weeks for development from egg to adult.

In our experiments two methods were used for obtaining blood from the bugs. For samples of pure blood, the legs and antennae were cut and the exuding droplets taken up in a pipette and diluted with physiological (0.85%) saline solution. This is a time-consuming procedure. For routine testing, therefore, the bugs were slit along the thorax and abdomen and the blood extracted by shaking in saline solution. This extract was filtered before use. When distilled water was substituted for saline solution, the extract was devoid of activity.

There are two possible objections to obtaining blood by the rapid extraction method: first, the solution thus obtained is heavily contaminated with bacteria; second, there is the possibility of contamination with feces of the bugs. With respect to the first objection, our observations lead us to believe that the bacteria thus introduced contribute nothing to the antibacterial action of the extract, since the extract may be put through a bacteriological filter or may be boiled up to 30 min without losing its activity. With respect to the second objection, it may be noted that tests with blood obtained without fecal contamination gave exactly the same results as those utilizing the extract. Further, if adult bugs were used, they either defecated on being slit, thus enabling removal of the feces, or usually they did not defecate at all. With nymphal bugs, which often defecated in the solution, fecal contamination was a factor, reducing the antibacterial activity. For this reason, adult bugs were used for all the experiments except those designed specifically to test the blood of nymphal bugs.

TABLE 1

Preparation	Diameter of ring of growth inhibition (mm)
1 part blood : 1 part saline	22
1 part blood : 49 parts saline	15
Filtered extract (1 bug/ml)	18–22
Same extract ether extracted, boiled, and refiltered	18–22
1 unit of penicillin/ml of dilute phosphate buffer	19–22

Antibacterial action was tested by the cylinder-plate method used in penicillin assay, using 10 ml of standard nutrient agar seeded with about 0.1 ml of a 24-hr broth culture of the test organism and incubating at 37° C for 16–18 hrs. When penicillin assay agar enriched with glucose and yeast extract was used, the zones of inhibition were smaller and not clear cut.

With *Staph. aureus* as test organism, the results presented in Table 1 were obtained. These show that, by the extraction method, the antibacterial activity from a single bug is the equivalent of above 1 Oxford Unit of penicillin. The actual content of the bug is undoubtedly greater, for this method does not extract all the blood. The blood of last instar nymphal bugs has antibacterial action like that of the adults.

The extract was active against *Staph. aureus* (F & D 209) and, to a lesser degree, *B. subtilis* (F & D 558-S),

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and was inactive against *B. subtilis* (ATCC 6633), *Escherichia coli, Salmonella typhosa, Pseudomonas pyocyanea, Alcaligenes fecalis, Corynebacterium diphtheriae mitis, Klebsiella pneumoniae, Mycobacterium phlei,* and *Streptococcus durans.*

Further experiments on properties of the active principle in the blood have so far yielded the following information: It is water soluble and not extractable from an aqueous solution with ether or butanol. It is destroyed slowly in the filtered saline extract on standing at room temperature, most of the activity being lost within 6 hrs. This destruction is hastened by heating, most of the activity being lost after boiling for over 30 min or autoclaving at 20 lbs for 10 min. If the lipids are removed from the saline extract by ether extraction before boiling, however, the solution is more stable. The active agent is not a protein which is precipitated by boiling, for removal of the precipitated proteins after boiling the saline extract does not reduce the activity. Filtration of the extract through ordinary or bacteriological filters does not impair the activity.

Apparently the active agent is synthesized in the body of the bug and not found as such in the milkweed seeds, for hot and cold saline extracts of the seeds are inactive. Further, a diet of milkweed apparently is not sufficient to cause the production of this agent, for solutions of the blood of three other species of insects which feed on milkweed exclusively—the red milkweed beetle, *Tetraopes tetrophthalmus,* the harlequin milkweed caterpillar, *Euchaetias egle,* and larvae of the monarch butterfly, *Danaïs plexippus*—are inactive.

Antibacterial substances from the blood of insects have been reported previously, but these have not been so directly extracted or so similar to familiar antibiotics in action. Glaser (*1*) found the blood of a grasshopper destructive to bacteria pathogenic for grasshoppers, and Olivier (*3*) found neutralized aqueous NaOH washings of an acetone extract of macerated wax moth larvae, *Galleria mellonella,* active against tubercle bacilli.

The feces of blowfly maggots have also been found (*2, 4, 5, 6*) to destroy, even after autoclaving, such important pathogens as *Clostridium welchii, Salmonella typhosa, Brucella abortus,* and hemolytic streptococci. We have repeated the experiments of Simmons (*5, 6*) with feces from the larvae of the black blowfly, *Phormia regina,* using both his method and the cylinder-plate method of testing for antibacterial action against *Staph. aureus.* Our results have been uniformly negative. The observation of Gwatkin and Fallis (*2*) that antibacterial activity of the feces of maggots decreases regularly with rearing in captivity offers a possible explanation for the discrepancy in results. The flies we used have been reared for many years in the laboratory, for a time under sterile conditions. These facts suggest that the bacterial flora of the digestive tract of the maggots or of the larval food (*4*) may be involved in the production of the antibacterial agent.

Certainly, all of the results suggest that insects, famous for their hardiness and rapidity of reproduction, may be, directly or indirectly, sources of new antibacterial agents of possible practical value.

References

1. GLASER, R. W. *Psyche,* 1918, **25**, 39.
2. GWATKIN, R., and FALLIS, A. M. *Canad. J. Res.* (D), 1938, **16**, 343.
3. OLIVIER, H. R. *Nature, Lond.,* 1947, **159**, 685.
4. PICADO, C. *Bull. Biol.,* 1935, **69**, 409.
5. SIMMONS, S. W. *Bull. ent. Res.,* 1935, **26**, 559.
6. SIMMONS, S. W. *J. Bact.,* 1935, **30**, 253.
7. WORTHLEY, H. N. In *Chemical control of insects* (AAAS Publ. No. 20), 1943.

Protection of Mice Against an Encephalitis Virus by Means of Organic-Solvent Extracts of Brain Tissue

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It has been shown (*1*) that a material extracted from the sera of normally-appearing animals by means of organic solvents inactivated certain neurotropic viruses *in vitro,* and a substance having similar properties, although to a lesser degree, could be obtained from brain tissue. Since these materials were without pronounced toxic effect in mice, attempts were made to uncover any possible action on the viruses *in vivo.* As will be noted, tests with Russian Far East (spring-summer, tick-borne) encephalitis virus revealed protection in mice against small amounts of virus by means of such extracts of brain tissue.

Brain extracts. Brain tissue from either apparently normal mice or sheep or from mice infected with viruses unrelated to the Russian virus was extracted in succession with acetone and ethyl ether or with a mixture of chloroform and methyl alcohol. The extracts were filtered through filter paper, evaporated to dryness, resuspended in saline solution, and dialyzed against 5% of 0.15M phosphate buffer in saline solution, pH 7. The final volume of the suspension was four times the weight of the brain tissue extracted. The preparations were stored at 4° C, and their protective effect was evident even after heating at 62° C for 1 hr on 3 successive days or at 95° C for 1 hr once. Before injection into mice, these materials were centrifuged at 1,500 rpm for 15 min and the supernatant used. The treatment consisted of either two injections of 0.5 cc of this material given intravenously 2 and 24 hrs after or one 24 hrs before the intraperitoneal challenge inoculation of virus. Following injection of the extracts, the mice exhibited at times a mild reaction, with ruffled fur, decreased activity, and diarrhea; their appearance was normal within 24 hrs. Ten of 816 mice died, 7 of the 10 succumbing after receiving one preparation.

It soon became apparent that if this material were used within 10 or 12 days after preparation, it possessed