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.

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

JORDI CASALS and PETER K. OLITSKY

The Rockefeller Institute for Medical Research, New York City

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

no protective effect. If, however, the extracts were kept in the refrigerator for longer than 4 weeks—100 days is the longest period thus far tested—a definite and significant effect was noted.

Preparation of the extracts under strict aseptic conditions was not practical; it was found that in almost all cases a bacterial contaminant was present which appears to be in all instances of the *Alcaligenes* genus. The possible participation of this contaminant in the protective effect observed is being investigated. It has been found, nevertheless, that when the extracts shortly after preparation are sterilized by heating at 62° C for 1 hr on 3 successive days, they are as effective as the nonsterilized ones under similar conditions. In all instances Merthiolate in final concentration of 1:10,000 is added to each extract 2 days before it is used in a test.

Virus. Russian Far East encephalitis virus is the only one thus far used in these tests. It was assumed at the outset that an intracerebral challenge of resistance might be a test too severe to detect small protective effects; for this reason a neurotropic virus was chosen which could infect adult mice after peripheral injection, even in high dilutions. Decimal dilutions of virus were prepared, and 0.1 cc was given intraperitoneally to each mouse. In 25 titrations (including the 4 shown in Table 1) using from 8 to 24 mice/dilution, the control mice gave an average LD_{50} titer of $10^{-8.91}$, with a variance of 0.0788 and a standard deviation of 0.2808. If, following Lauffer and Miller, and Horsfall and Curnen (*2*), a difference of $2 \times \sqrt{2} \times$ standard deviation is considered as significant, any preparation which showed a difference of titer of virus in controls and in treated mice equal to or greater than 0.80 log unit should be regarded as significant. In 10 experiments thus far carried out, the differences of titer of virus in controls and in treated mice were as follows: −0.88, −0.93, −0.97, −1.23, −1.28, −1.33, −1.44, −1.46, −1.50, and −1.80 log.

Table 1 describes some of the tests. The mice were uniform in age, weight, and sex in each test. The controls were inoculated with dilutions ranging from 10^{-7} to 10^{-11}; the treated mice, in general, with dilutions of 10^{-7} to 10^{-9}. The LD_{50} is calculated on the assumption that when the 10^{-7} dilution of virus was not inoculated, all treated mice if given this dilution, would have died; also, had the treated animals received the 10^{-10} dilution, all would have survived; the latter is probable, since the untreated had with this dilution a death rate of only about 10%. It will be noted that the preparation employed in Experiment 1 failed to protect when it was kept in the refrigerator for only 2 days, but this preparation as well as the others, when "aged" from 4 weeks to 100 days, yielded a degree of protection that was, according to the standard here used, significant. It can further be shown that if large enough numbers of mice are used per dilution, the results are unlikely to occur by chance (Experiment 2); thus, for the 10^{-8} dilution, $p = 0.0004$, and for the 10^{-9}, $p = 0.000001$. No protection was detected when treatment was delayed 48 hrs after infection.

In conclusion, mice infected with Russian Far East encephalitis virus by intraperitoneal route can be protected significantly with a material extracted by means of organic solvents from mouse or sheep brain tissue. The brain extract, when aged 4 weeks or longer in the refrigerator, was given intravenously either 24 hrs before or 2 and 24 hrs after injection of the virus. In the tests reported the difference in titer of virus between the treated mice and controls ranged from −0.88 to −1.80 log, values which are significant.

TABLE 1

MICE INFECTED INTRAPERITONEALLY WITH RUSSIAN FAR EAST VIRUS AND TREATED INTRAVENOUSLY WITH MATERIAL EXTRACTED FROM MOUSE OR SHEEP BRAIN WITH ORGANIC SOLVENTS

Exp.	Brain extract in refrigerator (days)	Treatment and source of extract	When treated in relation to virus inj.	Dilution of virus					LD_{50}	Difference*
				−7	−8	−9	−10	−11		
1	2	None, controls		12/12†	11/12	7/12	1/12	0/12	9.14	
		2 inj. Prep. A, (mouse)	2 and 24 hrs after		12/12	5/12			8.86	− .28
	29	None, controls		12/12	11/12	6/12	0/12	0/12	8.93	
		2 inj. Prep. A, (mouse)	2 and 24 hrs after		2/12	2/12			7.70	− 1.23
2	100	None, controls		18/20	18/20	16/20	1/12	0/12	9.28	
		1 inj. Prep. B, (mouse)	24 hrs before	17/18	7/20	1/20			7.78	− 1.50
3	28	None, controls		14/15	15/15	8/15	1/10		9.07	
		1 inj. Prep. C, (sheep)	24 hrs before	9/15	2/15	0/15			7.27	− 1.80

* −.80 log or less is significant based on $2 \times \sqrt{2} \times$ standard deviation.
† 12 mice died of 12 inoculated.

In all instances Merthiolate in final concentration of 1:10,000 is added to each extract 2 days before it is used in a test.

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