atmospheric contamination either produced relatively low-lying clouds or vented small amounts of radioactive vapors and gases into the lower layers of the troposphere. Considering the small amounts of iodine-131 required to explain the highest observed levels of iodine-131 in fallout and the apparent ease in the selective venting of tellurium and iodine, there has been no lack of sources in the continental United States to explain most, if not all, of our iodine-131 fallout.

Prior to September 1961, when nuclear testing was resumed, the fallout of iodine-131 resulted principally from sources in the upper troposphere. The heavy fallout of radioactive debris over Troy, New York, on 25 April 1963, about 36 hours after the Simon explosion in Nevada (*4*), was largely due to a radioactive cloud with its base at an estimated altitude of 10 km. The fallout in southeastern United States in late September 1961 was similarly due to debris, initially in the middle or upper troposphere, carried down into surface layers of the troposphere by unusual meteorological conditions (*13*). Most radioactive debris in clouds in the upper troposphere remains there for an average of about 1 month. Thus short-lived radioactive products like iodine-131 decay substantially before falling out and are widely dispersed at low intensity.

For underground explosions and low-yield surface tests the fate of the radioactive products is less predictable. Radioactive products pass into the lowest layers of the troposphere where they can be deposited over a smaller geographical area within a few days. This increases the concentration of fallout and renders more hazardous the effects of short-lived radioactive products like iodine-131. Even underground tests which are largely contained below ground with only a limited release of radioactive gases and vapors cannot be overlooked as sources of iodine-131 fallout. Selective venting must explain the appreciable fallout of iodine-131 without corresponding increase in surface air radioactivity as in midwestern United States during May 1962.

Control of iodine-131 fallout will be more effective if we control its sources rather than the distribution and consumption of fresh dairy products. Better containment of underground tests will result from placing the shots at greater scaled depths (*30*), from avoiding areas of faulty soil structure, and from greater care in sealing access tunnels. Moreover, less damage will be caused by venting explosions if all tests are carried out during winter under favorable meteorological conditions so that iodine-131 is not deposited where dairy animals are at pasture.

The high frequency of venting of radioactive products from previous underground tests suggests that either there was no serious attempt to contain them, or that containment is difficult and uncertain. Atmospheric radioactivity from vented underground explosions may be detectable in the lower atmosphere at large distances and may be distinguished from that resulting from atmospheric tests or reactor accidents.

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31. I am indebted to Samuel Penn of the Air Force Cambridge Research Laboratories, Bedford, Mass., for computing the tropospheric air trajectories, Fig. 2, and for helpful discussion on the meteorological aspects of this paper.

24 October 1963

Cholinesterase Inhibition in Spider Mites Susceptible and Resistant to Organophosphate

Abstract. *Evidence is provided that organophosphate resistance in a strain of spider mites is due to decreased sensitivity of its cholinesterase to organophosphates. The cholinesterase activity of the susceptible strain in vitro was three times that of the resistant strain of mites.*

Cholinesterase and acetylcholine are known to occur in the red spider mite, *Tetranychus urticae* Koch (*1*). Inhibition of this enzyme is generally assumed to be the mode of action in organophosphate poisoning. For this reason the cholinesterase of the mite was studied in vitro and a strain resistant to several organophosphates was also studied, because resistance may theoretically be caused by a difference in some characteristic of the cholinesterase of susceptible and resistant strains.

The strains of *T. urticae* used were described by Helle (*2*). The resistant strain was obtained by repeated backcrossing with the susceptible strain,

and selection with parathion from a resistant strain, "Systox" (*3*). This resistant strain is largely identical with the susceptible strain except for its resistance which Helle (*2*) proved to be monofactorial and semidominant. Both the susceptible strain and the resistant strain "Systox" were obtained from Prof. Unterstenhöfer, A. G. Bayer, Leverkusen, Germany, and have been used by Voss, Dittrich, and Saba (*4*).

Spider mites at different stages of development, both males and females, or selected adult females, were homogenized in 0.1*M* pyrophosphate buffer containing 3 percent NaCl, at *p*H 8 and 0°C. The homogenate was disintegrated by applying high-frequency sound (30 kcy/sec) for 1 minute under nitrogen. The suspension thus obtained was centrifuged at 30,000*g* for 30 minutes, and the supernatant was used.

To determine the activity of cholinesterase, the method described by Ellmann *et al.* (*5*) was used, with acetylthiocholine as the substrate. The reaction was carried out in semimicro cells in a total volume of 0.7 ml. Final concentrations in the reaction mixture were as follows: supernatant of a homogenate of 1 mg of mites per ml; $3 \times 10^{-4}M$ acetylthiocholine; $3 \times 10^{-4}M$ 5,5-dithio-bis-2-nitrobenzoic acid; 0.1*M* pyrophosphate buffer, *p*H 8; and 3 percent sodium chloride. The optical density of the yellow, 5-thio-2-nitrobenzoic acid, produced by reduction of the reagent by thiocholine, was measured at 412 mμ. To prevent oxidation of the thiol compounds the cells were closed with rubber stoppers (*6*) and evacuated through an injection needle. The vacuum was replaced by nitrogen. The change in the optical density was recorded for about 1 hour at room temperature by means of a Beckman D.U. spectrophotometer.

With $3 \times 10^{-4}M$ acetylthiocholine the activity was normally inhibited by eserine, the homogenates of susceptible mites showing 50 percent inhibition at about $2 \times 10^{-7}M$ eserine. The cholinesterase activity in the supernatants obtained from the susceptible strain of mites was about three times that from the resistant strain. Similar results were found when whole homogenates were used, but in this case there was interference from oxidation of the thiol compounds produced during the reaction. The manometric cartesian-diver technique gave qualitatively similar results with $1.5 \times 10^{-2}M$ acetylcholine as substrate, but a rather high CO_2 production was found in the absence of the substrate.

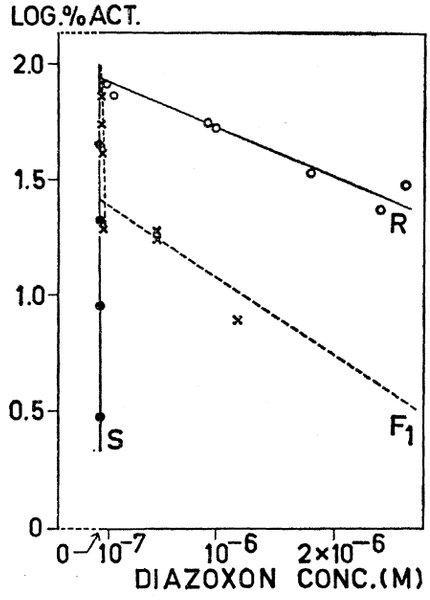

Fig. 1. The log of the percentage of cholinesterase-activity remaining after 30 minutes incubation with diazoxon at 27°C is plotted against the diazoxon concentration. ● Susceptible strain *S*; ○ resistant strain *R*; × hybrid of susceptible and resistant (F_1). The points represent single inhibition determinations obtained in two independent experiments.

With Hestrin's method (*7*) in which acetylcholine was used at $7 \times 10^{-4}M$, a *p*H of 7.5, and a temperature of 27°C, a threefold difference was again obtained. The acetylcholine was hydrolyzed at a rate of about 7×10^{-2} μmole per milligram per hour in the case of homogenates of susceptible mites. These results, obtained by three essentially different methods, provide strong evidence of difference between cholinesterase activities of the susceptible and resistant strains. The role of this enzyme in organophosphate poisoning and the use of a backcrossed resistant strain both suggest a causal connection between this difference and resistance. This suggestion led to a comparison of the sensitivities of the cholinesterases of susceptible and resistant strains to organophosphates. Experiments on cholinesterase inhibition were therefore conducted, with diazoxon (*3*) being added to the supernatants of the homogenates from both susceptible and resistant strains of mites, 30 minutes before addition of the substrate solution.

The results of two such experiments are shown in Fig. 1, and are plotted as the log of the percentage of activity against the concentration of the inhibitor; the results conform with pseudo-first-order kinetics. The mean bimolecular rate constants (k_2) were about 3×10^6 and 2×10^4 liter mole^{-1} min^{-1} for susceptible and resistant strains, respectively. Inhibition appeared to be constant during the assay and was uninfluenced by a 50-fold increase in the concentration of substrate. When paraoxon (*3*) was added instead of diazoxon, there was an even larger difference between the k_2 values, which were approximately 10^5 and 10^2 liter mole^{-1} min^{-1}, respectively, for susceptible and resistant strains. This finding is in harmony with the observation (*8*) that resistance to parathion is greater than to diazinon.

Thus, the organophosphates have considerably less effect on the cholinesterase obtained from the resistant strains, and it seems probable that the cholinesterase is the site of action in organophosphate poisoning. The low cholinesterase activity in resistant strains appears to be a side effect which seems to have no important consequences for normal maintenance of vital functions, because neither Helle (*2*) nor I have found any indications of decreased viability in the resistant strain. The difference in rates of inhibition would only delay death, unless the inhibitor was detoxified, or counteracted in some other way. It is assumed, therefore, that some mechanism, which both the susceptible and resistant strains of mites must have in common, has sufficient time to eliminate the poison in the resistant, but not in susceptible mites.

The results of two experiments on the inhibitive effect of diazoxon on the supernatants of adult F_1 females, being the mixed offspring of the reciprocal crosses between the susceptible and resistant strains, are shown in Fig. 1. Two rates of inhibition can be distinguished; roughly 75 percent of the activity shows a k_2 value of about 2×10^6, the remaining activity, a k_2 value of 3×10^4 liters min^{-1} mole^{-1}. These k_2 values, as well as the ratio between the corresponding activities, agree well with the results obtained for cholinesterase from the parent strains. The results with the F_1 supernatant prove that it is, indeed, the cholinesterase molecules that are different in the susceptible and the resistant strains. Since Helle (*2*) proved that the resistance is determined by

only one genetical factor, the difference in the rates of inhibition should also depend on this single factor.

Thus, the available genetic and biochemical evidence strongly supports the view that the mechanism of resistance in our resistant strains is a decreased sensitivity of the site of action of organophosphate poisoning, namely the cholinesterase. Furthermore, the rates of inhibition in the susceptible and the resistant strains appear to be controlled by two allelic genes.

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Dengue Types 1 and 4 Viruses in Wild-Caught Mosquitoes in South India

Abstract. *Three strains of type 1 dengue virus and two strains of type 4 dengue virus have been isolated from* Aedes aegypti *collected in Vellore, India.*

During investigations of dengue at Vellore in 1961, the dengue virus was isolated from 20 samples of human serum and from five samples of pooled *Aedes aegypti*. The viruses isolated from the human serum all proved to be type 1 dengue virus, while both types 1 and 4 were isolated from the mosquitoes. This is the first report of types 1 and 4 being found in mosquitoes and the second record of dengue viruses being isolated from wild-caught mosquitoes.

Although mosquitoes had been implicated in the transmission of dengue early in this century, it was not until 1960 that isolation of the virus from wild-caught mosquitoes was reported (*1*). Hammon *et al.* at that time described two new serotypes of dengue virus from the Philippines, types 3 and 4, and reported the isolation of type 3 from *Culex tritaeniorhynchus* as well as from *Aedes aegypti*. In addition, they reported the isolation of type 2 dengue virus from three samples of pooled *A. aegypti* in Bangkok. In 1960 (*2*), type 4 dengue virus was found in samples of human serum at Vellore, and previously types 1 and 2 had been isolated in the same locality (*3*).

Commencing in September 1961 with the onset of clinically diagnosed cases of dengue in Vellore, and continuing until the end of the rainy season in December, 77 pools of *Aedes aegypti* were inoculated into suckling mice. Both resting and biting mosquitoes were collected and combined in pools ranging in size from 1 to 55 mosquitoes, most of them containing between 15 and 30.

Groups of live mosquitoes, comprising one pool, were held overnight at room temperature prior to grinding with 1.5 to 2.0 ml of phosphate saline buffer containing 0.75 percent bovine albumin (BAPS) at *p*H 7.2, with penicillin and streptomycin added. Suspensions were centrifuged at 1500 rev/min for 20 minutes. The supernatant fluid was inoculated into two litters of infant mice, 0.02 ml intracerebrally and 0.03 ml subcutaneously, and a portion was stored at −50°C. Brains of sick mice were excised and suspended in BAPS (1:10). After centrifugation of this suspension, additional litters were inoculated.

Dengue viruses were isolated from five pools, each consisting of from 10 to 42 engorged mosquitoes collected on 10, 21, and 31 October and 3 and 6 November (strains 1300, 1318, 1328, 1332, and 1335). With strain 1328, most of the mice became ill and died after inoculation of the mosquito suspension, and the virus strain was readily established in successive passages through mouse brains. By inoculating mice with the original mosquito suspension after storing it for 10 days at −50°C, the virus could again be isolated successfully. With the four other virus strains, only two or three mice showed signs of illness out of each group of six inoculated. A virus strain was established in each instance; however, attempts to isolate the virus again from the original mosquito suspensions were unsuccessful. Routine passages from mice not showing signs of disease were not made. All strains passed a Seitz EK filter.

Table 1. Relationships between the viruses isolated from the mosquitoes and the dengue virus, types 1, 2, and 4, shown by complement fixation.

Antigen	Mouse immune serum (titer)				
	D1 82–1	D2 60–1	D4 968 1R	1318	1300
D1 82–1	*128*	32	16	128	8
D2 60–1	64	≥*512*	64	64	64
D4 968–1R	16	64	*256*	32	256
1318	64	8	8	*64*	<4
1332	64	8	8	32	<4
1335	64	32	8	128	4
1300	64	64	512	32	*256*
1328	32	64	256	32	128
Normal brain	<4	<4	<4	<4	<4
Veronal-buffered saline	<4	<4	<4	<4	<4

Incubation periods on initial passage were 9 and 10 days for two strains and 11 to 14 days for three. By the 20th passage, the incubation periods had become shortened to 4 and 7 days, respectively. Illness, after adaptation in mice, was characterized by tremors, wasting, and flaccid paralysis.

Complement-fixation (CF) tests with crude antigens in a single dilution (10 percent infected mouse brain suspension, 10th to 20th passage, in veronal-buffered saline) showed the three strains (1318, 1332, and 1335) with 7-day incubation periods to be type 1 dengue virus while the remaining two, with 4-day incubation periods (1300 and 1328) were type 4 (Table 1). For brevity, data on only two virus-homologous serum pairs are included. Hyperimmune serums were prepared by intracerebral or intraperitoneal inoculation of adult mice with live virus, followed by three intraperitoneal injections of live virus

Table 2. Neutralization of strains 1300 and 1328 by dengue 1, 2, and 4 immune sera prepared in mice.

Virus strain	LD_{50} virus dilution with mouse immune serum:			BAPS
	D1	D2	D4	
1300	10^{-4}	$13^{-3.5}$	10^{-1}	$10^{-6.4}$
1328	$10^{-4.4}$	$10^{-4.8}$	$10^{-3.4}$*	$10^{-6.3}$

* The D4 immune serum used in this test was less potent than that used with strain 1300.