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.

at weekly intervals. The reference antigens were from strains isolated from samples of human serum at Vellore, dengue 1 and 2 in 1959 (*3*) and dengue 4 in 1960 (*2*), they were identified either in Poona or at the Rockefeller Foundation Virus Laboratories in New York. In interpreting these results it should be noted that the antigens are crude and unstandardized. Cross hemagglutination-inhibition tests with either sucrose-acetone extracted or alkaline-aqueous antigens and mouse immune sera (*4*) confirmed the results obtained with CF tests.

The results of intracerebral neutralization tests in newborn mice with strains 1300 and 1328 are presented in Table 2. Neutralization tests with type 1 strains have been unsatisfactory so far because of low virus titers.

Four types of dengue virus have now been isolated from mosquitoes. With but one exception all isolations have been from *Aedes aegypti,* and our own investigations have been limited to this species. In addition to the three closely related dengue viruses active in Vellore, two other group B arthropod-borne viruses, Japanese encephalitis and West Nile, are found in this area (*3*).

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Antibody Suppression by Antigen Heavily Labeled with Iodine-131

Abstract. *Antibody formation in rabbits was suppressed by tobacco mosaic virus heavily labeled with iodine-131. Animals were injected intravenously with virus containing 0.5 to 4.4 mc of* I^{131} *and after a period of 6 days were challenged with a new antigen,* γ*-globulin. Most irradiated animals did not produce detectable antibody. No signs of radiation sickness were noted.*

In recent years the inhibition of immune responses by irradiation has proven valuable in experimental tissue transplantation studies. Congdon and Urso (*1*) performed successful bone-marrow transplantations in mice using external irradiation to suppress rejection. Others have obtained similar results (*2*). In larger animals and also in humans this method has not been satisfactory. The use of external irradiation in man to allow kidney transplantation produced serious effects on the gastrointestinal tract which led to hemorrhage and diarrhea (*3*).

Table 1. Ratio of radioactivity (counts per minute) in organs to that in muscle.

Period				
1 hr	1 day	2 days	5 days	8 days
		Marrow		
			12	138
		Spleen		
166	1681	189	115	652
		Mesenteric nodes		
		4.0	5	3.4
		Thymus		
		0.134	1.15	0.61
		Liver		
137	657	68	16	149
		Blood		
2.0	5.4	0.56	4.8	1.8
		Lungs		
23	39		3.5	6.1

This report concerns a feasible method for irradiating only those tissues participating in the production of antibodies by the administration of antigens which have been heavily labeled with a radioisotope. The method depends upon selective concentration of antigens and their proximity to the cells which initiate production of antibodies.

Wissler (*4*) has described a series of cytological changes in the reticuloendothelial system of the rat after administration of typhoid vaccine. The antigen is trapped by the phagocytes of the spleen, and the nearby primitive reticular cells then begin to proliferate. Perhaps these cells are stimulated by the antigen that has become soluble, since the reticuloendothelial cells have a mechanism which renders soluble both particulate foreign or domestic matter. Finally, numerous small, dark staining cells are formed which move out into the body and presumably participate in the antibody production process.

The site of action of radiation in suppressing antibody formation has not been established. Salerno and Friedell (*5*) compared the effects of external and internal irradiation. Colloidal Au^{198} and $CrP^{32}O_4$ were injected intravenously into rats and the change in antibody titers to sheep red cells was followed. Colloidal gold suppressed the antibody production somewhat, but the dose was lethal before the degree of suppression of antibody was as great as that achieved by total body external irradiation. In this case, most of the radioactivity was in the macrophage cells; and there could be no transfer to the antibody-forming cells by digestion, and so forth.

Yttrium-90 in a chelated form has been used for selective irradiation of the immune system (*6*). It was distributed throughout the extracellular fluid and it was concentrated in lymph nodes to twice the amount of that in other sensitive organs, such as the intestinal mucosa. A selective depression of lymphocytes resulted.

The area of irradiation in tissue is dependent upon the range of the particles at the site. If the cell in which the labeled antigen is localized is the only cell where radiation is desired, then an emitter, a substance emitting very soft beta rays such as H^3, or one emitting alpha rays such as astatine-211, is desirable. The alpha particle of astatine has a path length of about 60 μ. If antibodies are produced in other nearby cells, I^{131} would be preferable for irradiation because it emits the longer range beta particles. The most energetic beta radiation (0.6 Mev) has a maximum path of 2 mm in tissue, and the isotope is readily attached to protein.

Several of the many antigens that can be labeled with a radioactive isotope were studied: there was wide variation of the rate of disappearance from the host's serum. Bovine serum albumin injected intravenously into rabbits stays in the general circulation for days. If such a substance were used, a considerable proportion of the total dose would be delivered to the whole body. On the other hand, particulate antigens such as heat-killed typhoid bacillus disappear from the circulation. Tobacco mosaic