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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14 November 1963

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

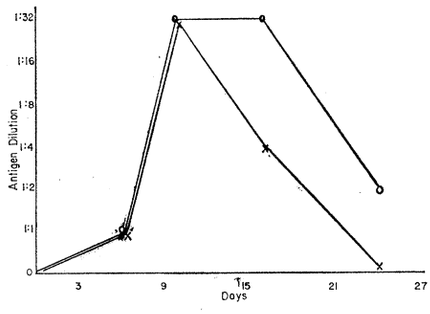

Fig. 1. Antibody response of irradiated animals. Ordinate, dilution of HGG from standard solution containing 1000 μg per milliliter; abscissa, days after challenging dose of HGG; (+) rabbit receiving 1 mc I^{131} TMV; (0) rabbit receiving ½ mc I^{131} TMV. Five animals, receiving 1.4, 1.6, 2, 2, and 4.4 mc, showed no antibody response and are not represented.

virus (TMV) very rapidly disappears (*7*) in a matter of minutes. Because of its ready availability and the rapid serum clearance this antigen was selected as the isotope carrier.

Tobacco mosaic virus (*8*) of the U-2 variety, maintained in cacodylate buffer, was dialyzed in 4-mg amounts against a solution of borate buffer at *p*H 8.0 and I^{131} was introduced by the iodine monochloride method (*9*).

Circulating TMV was detected by withdrawing samples of blood from the rabbits, performing a precipitin test on the separated serum, and measuring the radioactivity (due to I^{131}) in the washed precipitate. Within 1 hour, less than 10 percent of the TMV remained in the serum.

Five male Dutch rabbits were injected intravenously with 4 mg of TMV labeled with 5 μc of I^{131}. The animals were killed at intervals up to 8 days after injection. Samples of various organs were obtained and the I^{131} concentration was compared with that in the muscle in the same animal (Table 1). With respect to time, no definite pattern was seen. The relatively high concentrations in spleen, liver, lungs, and marrow are significant. A series of seven doses containing 0.5, 1.0, 1.4, 1.6, 2.0, 2.2, and 4.4 mc, respectively, was injected intravenously into the ear vein.

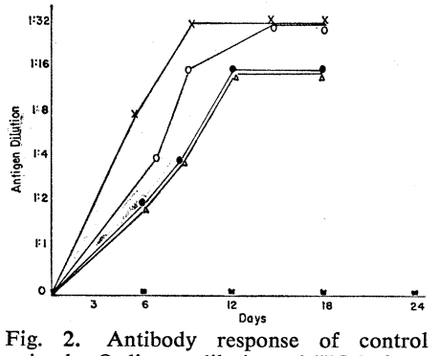

Fig. 2. Antibody response of control animals. Ordinate, dilution of HGG from standard solution containing 1000 μg per milliliter. Abscissa, days after the challenging dose of HGG. Each line represents antibody response of an individual animal. One animal showed no antibody response.

A total-body count was performed on one treated rabbit. Very little radioactivity remained after 10 days; it decreased during the first day to 40 percent and in the next 10 days to 10 percent.

Antibody titers to TMV were determined by the capillary-tube method in two of these animals on the 7th day after injection. No depression was seen or expected, since the major part of the irradiation should have been delivered several hours after administration of the I^{131}-TMV. According to Taliaferro (*10*) the radiation effect is most pronounced when the radiation is delivered one day prior to the antigen. At 6 to 7 days after I^{131}-TMV was given, the capacity to produce antibodies was tested by the administration of another antigen, human γ-globulin (HGG). Antibody titers to this test antigen were determined by the ring test. A cloudiness at the interface of the antigen layered on the antiserum within 2 hours was considered indicative of antibodies. After 7 days, all animals failed to produce detectable antibodies except those animals receiving 0.5 and 1.0 mc of I^{131}-TMV (Fig. 1).

All animals appeared to be in excellent health during the experiment. They ate well, gained weight, and showed no overt signs of illness.

The experiment was controlled by five Dutch rabbits from the same group to which TMV iodinated with nonradioactive iodine was given. They were given the test antigen in the same manner. All of these animals but one produced detectable antibodies to the test antigen (Fig. 2).

Although the number of animals tested was fairly small, there was a definite suppression of antibody formation detectable by the test described.

A very significant point is that no definite signs of radiation sickness or death occurred in these animals.

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8 October 1963

Ethacrynic Acid: Diuretic Property Coupled to Reaction with Sulfhydryl Groups of Renal Cells

Abstract. *Ethacrynic acid, injected intravenously after surgical removal of one kidney of anesthetized dogs, lowered the concentration of protein-bound sulfhydryl groups in cells of the remaining kidney. Chloride excretion and urinary output of the kidney exposed to the drug increased. These findings indicate that the diuresis produced by ethacrynic acid may be related to its capacity for binding sulfhydryl groups of renal cellular proteins.*

Ethacrynic acid (Fig. 1) is a remarkable diuretic drug in dogs and humans. The drug and its biologically active congeners react with sulfhydryl groups in vitro (*1*). Moreover, effects of ethacrynic acid on urine flow and sodium chloride excretion closely resemble the effects of mercurial diuretics (*2*), compounds known to react with protein-bound sulfhydryl groups of renal cellular cytoplasm (*3*). These observations indicate that ethacrynic acid and mercurials may act in similar ways. As a first step in determining the way in

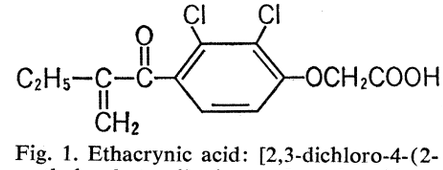

Fig. 1. Ethacrynic acid: [2,3-dichloro-4-(2-methylenebutyryl) phenoxy] acetic acid.