shock.[1,2,3] The modifying action of these drugs again seems in accord with the theory that histamine plays a major role in anaphylaxis. It is of interest that approximately the same degree of protection was offered, in these experiments, by both the compounds used.

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Immunization of Mice Against Viruses of the Psittacosis Group with Ultraviolet-Inactivated Vaccines.*

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Various workers have demonstrated[1-4] that formalinized suspensions of psittacosis virus possess antigenicity as judged by their capacity to elicit active immunity in mice and avian species. No report has appeared, to our knowledge, of the effect of ultraviolet rays on this or other viruses of the psittacosis group. Levinson, Milzer, and co-workers, employing a new method of inactivating turbid suspensions of viruses and bacteria by ultraviolet rays, have found that it is possible to prepare experimental vaccines[5-7] with dysentery bacilli, and rabies, St. Louis encephalitis, and poliomyelitis viruses with a minimal loss of antigenicity. Briefly, this technic consists of exposing a continuously flowing thin film of suspension to the Oppenheimer-Levinson type lamp which is a powerful source of total and extreme (below 2000 Angstroms) ultraviolet energy. This method of inactivation was employed in the present study with the viruses of psittacosis (6BC), human pneumonitis (SF), and ornithosis (207).

Previous experiments[8] have indicated that these viruses can be completely inactivated by this method and still retain antigenicity as determined by the production of neutralizing antibody in chickens repeatedly inoculated with these preparations. It was our purpose here to make a preliminary investigation of the possibility of using such preparations for the induction of resistance against challenge injections of active virus.

Materials and Methods. The strains of virus employed have been used in previous work in this laboratory and their sources have been recorded elsewhere.[9] Earlier studies[10-12] have shown that large amounts of virus in relatively clear suspensions can be obtained by harvesting the allantoic fluid from embry-

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1 Bedson, S. P., *Brit. J. Exp. Path.*, 1938, **19**, 353.

2 Yanamura, H. Y., and Meyer, K. F., *J. Immunol.*, 1942, **44**, 195.

3 Meyer, K. F., Eddie, B., and Yanamura, H., *J. Immunol.*, 1942, **44**, 211.

4 Morgan, H. R., and Wiseman, R. W., *J. Infect. Dis.*, 1946, **79**, 131.

5 Shaughnessy, H. J., Milzer, A., Neal, J., and Levinson, S. O., *J. Infect. Dis.*, 1946, **78**, 69.

6 Levinson, S. O., Milzer, A., Shaughnessy, H. J., Neal, J. L., and Oppenheimer, F., *J. Immunol.*, 1945, **50**, 317.

7 Milzer, A., Oppenheimer, F., and Levinson, S. O., *J. Immunol.*, 1945, **50**, 331.

8 Francis, R. D., to be published.

9 Hilleman, M. R., *J. Infect. Dis.*, 1945, **76**, 96.

10 Eaton, M. D., Martin, W. P., and Beck, M. D., *J. Exp. Med.*, 1942, **75**, 21.

11 Williams, S. E., *Aust. J. Exp. Biol. and Med. Sci.*, 1944, **22**, 205.

12 Francis, R. D., and Gordon, F. B., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 270.

onated eggs inoculated into the allantoic cavity with certain members of this group. Consequently, this method of cultivation was utilized in the preparation of agents used as vaccines in the present work. Infected allantoic fluids were pooled, filtered through sterile gauze pads and stored in the dry ice-box until needed. Immediately prior to irradiation, the frozen materials were thawed at 37°C and filtered again to remove the small amount of insoluble flocculent precipitate frequently present.

In order to determine the least amount of irradiation necessary to inactivate the agents completely, an allantoic fluid preparation of each virus was divided into several portions which were irradiated for different periods of time. The exposure periods employed varied by 0.05-0.10 seconds and ranged from 0.05-0.30 seconds. It was found that periods of 0.10, 0.15, and 0.20 seconds were required to inactivate our preparations of psittacosis, human pneumonitis, and ornithosis viruses, respectively. A larger quantity of each virus was then titrated by intracerebral inoculation of mice, and inactivated by exposure to ultraviolet irradiation for the minimal time necessary as determined by the preliminary trials. Complete inactivation was confirmed in each instance by the tests described below. The LD_{50} titers,[13] by intracerebral test, before irradiation of the preparations finally used for immunization, were 0.03 cc x $10^{-6.3}$, $10^{-6.5}$, and $10^{-5.8}$, respectively, for the viruses of psittacosis, human pneumonitis, and ornithosis. Inactivated virus was stored in the dry ice-box, without the addition of a preservative, until used for the immunization of mice.

Rigorous tests for complete inactivation of irradiated virus were employed. They consisted of 3 serial intracerebral passages in mice and 3 serial allantoic passages in embryonated eggs. If these passages were all negative for active virus, 3 serial passages were performed by the intranasal route in mice and by the yolk sac route in embryonated eggs. In every instance, when active virus was present, as in the preliminary trials, it was demonstrated in either the first or second serial intracerebral or allantoic passage. Evidence suggested that the intracerebral route in mice and allantoic route in eggs were equally sensitive in the detection of active material.

Mice were vaccinated intraperitoneally with 2 and 3 0.5 cc injections of undiluted irradiated virus given at 5- or 7-day intervals. The challenge dose of homologous virus consisted of suspensions of infected mouse brain or pooled liver and spleen tissue, and was given either intracerebrally or intraperitoneally to groups of mice together with controls, 3 weeks after the terminal injection of vaccine. The LD_{50} titers of these suspensions were determined before use by intracerebral or intraperitoneal inoculation of mice, depending upon the tissue source.

The emulsion of mouse brain passage virus, first used for intraperitoneal challenge of mice (those which received 2 injections of psittacosis vaccine, Table I) was not sufficiently virulent by this route to give regular, clear-cut results. An attempt was made to overcome this circumstance by performing several rapid serial intraperitoneal passages with emulsions of pooled virus-infected liver and spleen tissue. A definite enhancement in virulence was obtained after 8 passages as indicated by a decrease from 7 to 2 days in the average survival time of animals inoculated with 10% emulsions. An emulsion of pooled infected liver and spleen tissue was then prepared, titrated, and used as the challenge inoculum in the experiment where 3 injections of psittacosis vaccine were employed.

Results. The results shown in Tables I and II indicate that a definite degree of protection was demonstrable in most experiments. Little or no resistance was seen in mice challenged intracerebrally with psittacosis virus (Table I), but definite immunity was demonstrated in psittacosis-vaccinated mice when the challenge dose was given intraperitoneally. Evidently this resistance was not due in part to non-specific immunity since no protection was seen in mice which had received three intraperitoneal injections of normal allantoic fluid three weeks before the challenge dose of

[13] Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.

TABLE I
Resistance of Mice Immunized with Ultraviolet-Inactivated Psittacosis Virus.

Injections of vaccine	Challenge Injection			Result†	
	Volume	LD_{50} Doses	Route	Vaccinated mice	Non-vaccinated controls
2	0.03 cc	4,000	Intracerebral	12/12	6/6
"	"	400	"	12/12	6/6
"	"	40	"	12/12	6/6
"	"	4	"	6/12	6/6
"	0.03 cc	40,000	Intraperitoneal	2/3	6/6
"	"	4,000	"	0/10	4/6
"	"	400	"	0/10	4/6
"	"	40	"	0/10	4/6
3	0.25 cc	48*	"	15/38	27/27 26/26**

†Numerator indicates number of deaths; denominator indicates number of mice inoculated.
*LD_{50} titer determined here by intraperitoneal inoculation; all others determined by intracerebral.
**This set of controls received normal allantoic fluid at times of immunization.

TABLE II
Resistance of Mice Immunized with Ultraviolet-Inactivated Human Pneumonitis and Ornithosis Viruses.

Virus	LD_{50} Doses of Challenge Injection (0.03 cc, intracerebral)	2 inj. of vaccine		3 inj. of vaccine	
		Vaccinated	Control	Vaccinated	Control
Human Pneumonitis	180	5/8	8/8	4/10	8/8
	18	0/7	8/8	0/10	8/8
Ornithosis	900			8/10	6/6
	90	3/9	6/6	1/10	6/6

virus was given. Resistance to intracerebral challenge was definitely demonstrated in mice which had received human pneumonitis and ornithosis vaccines (Table II).

Conclusions. These studies demonstrate that it is possible to inactivate completely certain viruses of the psittacosis group in less than one second by exposure to appropriate intensities of ultraviolet irradiation. Such preparations retain antigenicity as indicated by their ability to immunize mice against challenge doses of active virus.

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Techniques for Rickettsial and Virus Cultivation.

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The following note describes modifications made in the Weigl method for cultivating rickettsiae and other small agents of disease within susceptible lice. Made primarily to facilitate isolations from diseased persons, they involve feeding larvae reared under sterile conditions on potentially infected blood.

The technique is essentially as follows. From 200-400 adult lice (*Pediculus vestimenti*) are washed for 2 minutes in 60% alcohol and subsequently with sterile physiological saline. They are then enclosed in a cage (6 x 3 x 1 cm) covered on one side with a fine net and containing within it the piece of