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.

JOZEF KUBICZ. (Introduced by R. W. G. Wyckoff.)
Wroclaw, Poland.

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

cloth recommended by Weigl. The caged lice are fed twice daily on a healthy nourisher whose skin is first sterilized with 70% alcohol. Between feedings the cages are kept in sterile paper boxes at 34°C. Under these circumstances, masses of eggs are laid on the cloth. These eggs are removed and washed first with 30% alcohol or other suitable weak disinfectant and then with sterile saline, bacterial sterility of the washed eggs being checked by culturing the last drops of the saline wash on nutrient agar. The sterile eggs are dried on sterile paper and incubated at 34°C in sterile net-covered cages, 200-400 eggs per cage. The eggs hatch after 4 to 6 days and the larvae are then ready for use.

When used to isolate rickettsiae or other agents of disease from a patient, such a cage of larvae is fed sterilely and twice daily over a period of four to twelve days, depending on the disease being investigated. To do this, the net side of the cage is attached to the sterilized skin on the medial side of the forearm or shank. Sterilization is affected with ether followed by 70% alcohol, care being taken to be sure that the alcohol has completely evaporated before feeding begins. Bacterial sterility is routinely checked by seeding larval excrement to agar plates.

At the conclusion of the 4- to 12-day feeding period, the cage is opened and note made of the living and dead larvae, especial attention being given those that are red or red-black in color. Infectivity is sought in the accumulated excrements and in the sterilely resected intestines of the living and dead reddish larvae. Microscopic examination is carried out with the aid of contrast methods of Serkowski[1] (Nigrosin, 4% in H_2O) or by the Eisenberg[2] method.

In this way, we have isolated and observed the infectious bodies responsible for a number of virus and rickettsiae-like diseases. These bodies are, for the most part, round disks having about the size of typhus rickettsiae. Several of the diseases they produce are rheumatic in character (*Polyarthritis rheumatica acuta, Chorea minor*), though one gives a toxic diarrhea in infants. To identify what is seen in stained preparations with the diseases in question, we have made use of both agglutination and of the Bordet-Gengou reaction. Some isolations have produced symptoms in rabbits and sensitized guinea pigs. Inactivated suspensions have given as intracutaneous test prominent urticaria-like reactions in persons sensitized through chronic disease and have thus proved a useful means of diagnosis. In sensitive persons, this reaction also supplies an excellent test for the presence of traces of the agents in louse intestines. At all times, corresponding emulsions of healthy louse intestines have been used as controls.

An essentially similar procedure for isolating infectious material has used bed bug (*Climicis lectularii*) instead of louse larvae. It often permits the isolation of agents that would be missed using louse larvae. In this case, small pieces of wood replace cloth as receivers of laid eggs and feeding is once a day for an hour. Each cage is set up for 60-80 eggs. Incubation takes place at a lower temperature (28-30°C).

This work was begun in the Typhus Institute of Professor R. Weigl and in the Children's Clinic of Professor Fr. Groer in Lwow. It has been continued in the Dermatologic Clinic of Professor Lenartowicz in Wroclaw.

1 Sterling-Okuniewski, *Technika badan bakteriologicznych* (Warzawa, 1922), p. 72.

2 Eisenberg, Ph., *Zentr. Bakt. I akt Originale Bd.*, **71**, 421.