THE INHERITANCE OF ACQUIRED IMMUNITY

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In 1927 Metalnikov published *L'infection microbienne et l'immunité chez la mite des abeilles* Galleria melonella. Amongst his many interesting experiments was a paper on the much discussed problem of the inheritance of acquired immunity.

Metalnikov immunised the larvae of the moth *Galleria* (which lives on honeycomb) with a heated culture of cholera bacillus, and in each subsequent generation he reimmunised a small percentage to serve as the parents of the next generation. The rest were reinfected with the live cholera bacillus, and after a few days the survivals were counted; the results obtained are shown in Table I.

Table I.

Generation	% survivals	Generation	% survivals
1st	0	7th	75
2nd	0	8th	72
3rd	30	9th	75
4th	16	10th	77
5th	0	11th	60
6th	42	12th	48

In 1931 Prof. MacBride suggested that the experiment should be repeated. As it was not possible to use cholera in a general laboratory, *Bacillus Danzy* was employed, the cultures of which were kindly supplied by the Lister Institute. This bacillus was frequently used in experiments with *Galleria* by Metalnikov (*vide L'infection microbienne et l'immunité*).

A stock of *Galleria melonella* was built up from six pupae provided by the Field Station at Slough. It was found that they throve best on old bee comb; if kept at 37° C. they completed their life cycle in 6–7 weeks.

Both the *Galleria* and *Bacillus Danzy* were kept in an incubator at 37° C. The amount of *Bacillus Danzy* injected each time was standardised as follows. 1 c.c. of sterile normal saline was added to a 24-hour culture of *Bacillus Danzy* on agar, and was well shaken to form an emulsion. Three drops of the emulsion from a graduated pipette were mixed with 1 c.c. of normal saline. 10 c.mm. of this dilution were found by experiment to be the minimum lethal dose. In a previous series of experiments (which was cut short by an accident to the incubator), two drops of emulsion were used; as there were frequent survivals in the controls, a stronger dose was employed in the next set of experiments.

The results obtained after inoculation were as follows: on June 7th, 1932, twenty caterpillars were injected with 10 c.mm. of the living culture (two drops of emulsion in 1 c.c. of normal saline). On June 22nd two normal moths were ob-

tained. On June 17th thirteen caterpillars were inoculated in a similar manner and on July 6th three moths were obtained. Again, on October 14th thirty caterpillars were injected with 10 c.mm. (three drops of emulsion in 1 c.c. of normal saline); on October 17th all these larvae were dead.

A vaccine was prepared by heating the culture of bacilli to 60° C. for 30 min.: it was always freshly prepared. It was found that an inoculation of 10 c.mm., an amount often used by Metalnikov, gave immunity. The 10 c.mm. of fluid were introduced into the body cavity of each caterpillar at one side between the 5th and 6th abdominal segments; in this way no important organ was punctured. The injection was made with a glass needle manipulated by a Chambers' micro-injection apparatus under a binocular microscope. The caterpillars were always injected in the last instar, as it was found that injection usually caused pupation, and if the grubs were not fully grown, the procedure produced immature moths. In the same way the immunised grubs were reinjected within 48 hours, before they had time to pupate. The results obtained were as follows:

On November 18th, twelve caterpillars were injected with 10 c.mm. of vaccine (three drops of emulsion in 1 c.c. of normal saline and heated to 60° C.). On November 19th the same caterpillars were injected with 10 c.mm. of live bacillus culture. On December 3rd twelve moths were obtained. Twelve non-immunised caterpillars injected on November 19th with living bacilli as controls were dead on November 22nd. It was found that the technique incidental to immunisation, viz. exposure of the caterpillars to room temperature, did not influence the percentage of emerging moths.

The possibility of hereditary transmission of immunity was investigated in four experiments, as follows:

I*a*. Fifteen fully grown caterpillars from one pair of moths were immunised with 10 c.mm. of fresh vaccine and placed in a jar with old bee comb; in about 6 weeks a new generation F_1 was ready for injection. Fifteen of these, chosen at random, were immunised with 10 c.mm. of vaccine and put into the incubator as parents of F_2 generation.

Fifty to 100 of the F_1 generation were injected with 10 c.mm. of live *Bacillus Danzy* (dilutions given above), and an equal number of controls (*i.e.* caterpillars from non-immunised parents) received the same dose. Twenty-four hours later the survivals were counted. This was continued to the ninth generation.

I*b*. Fifteen caterpillars from general stock were treated in exactly the same manner as I*a*.

II*a*. Fifteen caterpillars, the offspring of one pair of moths, were immunised with the 10 c.mm. vaccine. Twenty-four hours later they were injected with 10 c.mm. of live bacillus; fifteen controls were also injected to show that a lethal dose was being used; in each case all the immunised grubs lived but none of the controls lived for more than 3 days. The immunised and injected caterpillars formed the parents of the F_1 generation. As each generation was ready, fifteen caterpillars were immunised and injected and segregated to function as parents of the subsequent generation.

II*b*. Fifteen caterpillars from the general stock were treated in a similar manner.

Exp. I differed from Exp. II in that I*a* and I*b* were immunised only, while II*a* and II*b* received the vaccine and the live bacillus.

In each generation 20–100 caterpillars from each group (*i.e.* 80–400 altogether) were tested for immunity, an equal number of controls being used in every case.

Any caterpillars from immunised parents not tested in each generation were destroyed.

The number of caterpillars in each generation varied from 50 to 300, depending on the sex of the fifteen parent moths.

The results of these four sets of experiments are shown in Table II.

Table II.

	Series							
	I*a*		I*b*		II*a*		II*b*	
Generation	Survivals	Total used	Survivals	Total used	Survivals	Total used	Survivals	Total used
1st	0	20	0	20	0	20	1	20*
2nd	0	20	0	20	0	20	2	40
3rd	0	40	0	40	0	40	0	40
4th	1	30	0	40	0	10	1	40*
5th	0	40	0	40	1	50*	—	—
6th	0	20	0	50	0	50	—	—
7th	0	30	0	30	0	30	—	—
8th	1	100*	—	—	Died out	Died out	0	100
9th	1	100*	1	80*	—	—	1	100†

* In these instances one moth emerged from the controls which had been inoculated with living bacilli only: the number of the controls was in each case the same as the number of individuals inoculated with vaccine.

† Two controls survived.

In Expts. II*b* 5th, 6th, 7th there were only enough grubs to form the parents of the subsequent generations.

From the above results there appears to be no sign of inherited immunity. These results thus fail to corroborate the experiments of Metalnikov. This may be due to the use of a different bacillus, immunity to which takes longer to acquire, or a stronger culture may have been employed which required a greater number of generations before inherited immunity appeared.