then, we have shown that the improvement in first generation growth is due principally to iron and secondarily, but importantly, to zinc. A manganese deficiency can be detected in generation I, but becomes clearly apparent in generations II and III. Copper, at all dosages tested, has proved inhibitory, as are both iron and manganese at levels much above the optimum. The culture presently in its 20th generation is being reared on a diet which differs little from those employed for the past 3 years but for the addition of the foregoing trace elements and greater care in storing it between make-up and experimental use. The composition of this diet is given in the Table.

There are several points of particular interest in this result. Second generation weights at adulthood are considerably lower than in the first generation – 300 µg as opposed to about 500 µg – but thereafter growth appears quite stable, each generation of larvae taking about 10–12 days to become adult at 300–350 µg average weight. If anything, the most recent generations (VII–XX) have been rather larger and more fecund, we think as a result of increasing the level of zinc in recent lots of diet. With 20 successive generations, we probably have achieved a permanent culture on synthetic diet.

Generally we have only reared from *apterae*, because, in previous work, the larvae of *alatae* have tended to be feebler than those of *apterae*. However, recently we kept larvae deposited by generation IX *alatae*, and these grew as well as larvae deposited by *apterae* of the same generation.

An important point that emerges from this concerns lipid requirements. Our diet contains no sterol or any other lipid, and it seems clear, therefore, that dietary lipids are not needed by *Myzus*. The possibility that lipid reserves carried over from the original plant-reared mothers might still be involved seems far-fetched when it is realized that, with a weight increase of approximately twelvefold per generation, the dilution factor for original reserve materials must now, in the 20th generation, be of the order of $12^{20}$. If a dietary sterol is not required, one has to suppose either that *Myzus* is unique amongst insects so far studied in being able to synthesize sterol, or that its symbiotes provide it with sterol. We incline to the latter case.

Since the diet would appear, now, to be qualitatively complete, why then are our aphids smaller than aphids reared on plants (and, incidentally, very differently pigmented)? Of the 2 possibilities that come to mind, chronic suboptimal feeding or nutrient imbalance, we think we can dispose of the former. Uptake from diet, measured directly by difference weighing of sachets, is probably as good as from a plant: all instars consume 3–4 times their weight of diet per day, a value which compares well with those cited for other leaf-feeding aphids in the literature[6], and with our own estimates of uptake by plant-feeding *M. persicae*. Further, excretion is very similar to that of aphids on plants, frequency of honeydew droplet production being more or less identical. This, then, leaves nutrient imbalance; and we think that probably the area of imbalance is in the relative proportions of amino acids, perhaps in a limiting level of 1 or 2 only. If one supposes that a dietary imbalance were at first masked by reserves from the mother, and that growth thereafter were limited to a constant rate once such buffering of the dietary imbalance was removed by exhaustion of reserves, this would account for growth that was higher to begin with than in subsequent generations[7].

*Zusammenfassung.* Es gelang 20 Generationen der Pfirsichblattlaus, *Myzus persicae*, auf sich folgender steriler künstlicher Diät zu züchten.

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[6] J. L. AUCLAIR, A. Rev. Ent. *8*, 439 (1963).

[7] This work was supported in part by U.S. Public Health Service Grant No. A1 03497.

## Strain Influence on the Immune Response of Mice to the Friend Virus

In the past few years we have developed a new method called immunoelectroadsorption for the quantitative study of immunological reactions[1]. This method is based on the selective adsorption of antibodies on a slide coated with the corresponding antigen. It is found that the thickness of the layer adsorbed is greater when the antiserum is homologous rather than heterologous or normal. The thickness is measured optically with an ellipsometer[2].

By this method it was possible to demonstrate the appearance of antibodies against the Friend virus in NCS mice as early as 2 days after infection[3].

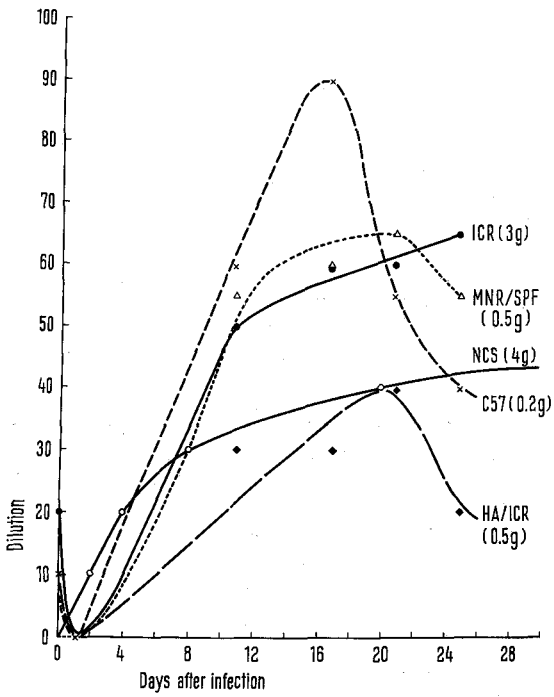
It was of interest to find out whether the strain of mice had any influence in the production of antibodies against the Friend virus; especially so, since it is known that certain strains such as MNR/SPF, C57 and HA/ICR are particularly resistant to the virus. In contrast, ICR and NCS strains are very susceptible to the disease. The results obtained are presented in this paper.

*Experimental.* The tests were conducted as previously described[1,2], the time for the adsorption of both antigen and antibodies was 30 sec and the intensity of the current 0.3 mA. 9 different stocks of mice were used. The mice were injected i.p. with 0.2 ml of a 10% saline suspension of infected spleen from NCS mice. The antigen used for all the experiments was prepared from a pool of infected spleens of the following stocks: HA/ICR, ICR, and Manor Farm SPF. For the antibody assay, blood samples were taken at regular intervals by orbital bleeding. Some of the most significant results obtained are summarized in the

[1] C. MATHOT, A. ROTHEN, and J. CASALS, Nature *202*, 1181 (1964).
[2] A. ROTHEN, Rev. scient. Instrum. *28*, 283 (1957).
[3] C. MATHOT, A. ROTHEN, and S. SCHER, Nature *207*, 1263 (1965).

| Strain | NCS | | NCS/D | | NCS souris | | NCS fauve | | C57 | | C3H | | HA/ICR | | ICR | | MNR/ SPF | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Weight of spleen, g | 4 | | 3.5 | | 3.5 | | 4 | | 0.2 | | 0.5 | | 0.5 | | 3 | | 0.5 | |
| Days after injection | Thickness layer A | Limiting dilution | Thickness layer A | Limiting dilution | Thickness layer A | Limiting dilution | Thickness layer A | Limiting dilution | Thickness layer A | Limiting dilution | Thickness layer A | Limiting dilution | Thickness layer A | Limiting dilution | Thickness layer A | Limiting dilution | Thickness layer A | Limiting dilution |
| 0 | 57 | – | 58 | – | 58 | – | 61 | – | 88 | 10 | 53 | – | 76 | 10 | 81 | 20 | 80 | – |
| 1 | | | | | | | | | 61 | – | | | 58 | – | 57 | – | 77 | – |
| 2 | 60 | – | 63 | – | 57 | – | 56 | – | | | | | | | | | | |
| 4 | 77 | 20 | | | | | | | | | | | | | | | | |
| 8 | 88 | 30 | | | | | | | | | 81 | 30 | | | | | | |
| 11 | | | | | | | | | 120 | 60 | | | 83 | 80 | 106 | 50 | 104 | 55 |
| 14 | 93 | 35 | 59 | – | 61 | – | 63 | – | | | 117 | 60 | | | | | | |
| 17 | | | | | | | | | 155 | 90 | | | 84 | 30 | 116 | 60 | 122 | 60 |
| 21 | 97 | | 61 | – | 57 | – | 59 | – | 103 | 55 | | | 93 | 40 | 118 | 60 | 130 | 65 |
| 25 | | | | | | | | | 90 | 40 | 165 | 80 | 75 | 20 | 123 | 65 | 110 | 55 |
| 28 | | | | | | | | | | | 95 | 50 | | | | | | |

Table and in the Figure. The thickness of the layer adsorbed following antiserum treatment increases with the concentration of antibodies but tends to a maximum as the concentration increases. A more accurate method to determine the titer of antibodies, is to dilute the antiserum until the thickness adsorbed is the same as that obtained with a serum containing no antibodies. The corresponding dilution is called the limiting dilution and is a measure of the concentration in antibodies. For the test all the sera were first diluted 1/10 in veronal buffer pH 7.5, 0.03 *M*. The figures shown in the Table represent either the thickness in Å units of the layer adsorbed after the antiserum treatment (column headed thickness layer) or the limiting dilution of the antiserum (column headed limiting dilution). When no figure is given in the column 'limiting dilution', it means that no measurable amount of antibodies was present in the serum diluted 1/10 in veronal. The limiting dilutions against the number of days after injection have been plotted for 5 different stocks in the Figure.




Antibody formation as a function of the time after injection as measured by the limiting dilutions of sera for 5 stocks of mice.

*Results.* The rate of production of antibodies following injection was approximately the same in the susceptible (ICR) and in the resistant (C57) strains. However, in the resistant strains the level of antibodies reached a maximum 18–22 days after injection and dropped sharply thereafter. By contrast, the antibody concentration in the susceptible strains ICR and NCS did not reach a maximum and kept increasing slightly with time. It was surprising to find that the stocks NCS/D, NCS souris, and NCS Fauve, which are all susceptible to the Friend virus, did not produce any detectable amount of antibodies, as it appears from the Table. It was also observed that, before injection, the sera of some of the stocks (C57, HA/ICR, ICR) gave an adsorbed layer roughly 20 Å thicker than that obtained with the other stocks. However, 1 day after injection the thickness of the adsorbed layer from these sera dropped to the value found for all the other stocks, that is within the narrow limit 53–60 Å.

To summarize, the immunoelectroadsorption method permitted the demonstration of the production of antibodies against the Friend virus in resistant as well as susceptible strains of mice. However, in the resistant strains the production of antibodies reached a sharp maximum within 3 weeks after injection, a course which was not observed with the susceptible strains.

*Résumé.* La méthode d'Immunoélectroadsorption a permis de déceler la présence d'anticorps contre le virus de Friend, non seulement dans les souches de souris résistantes, mais aussi dans les souches sensibles à ce virus.

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