

in 0.5% bovine albumin solution and centrifuged. The supernatant was treated in the same way as the plasma.

The Figure shows the plasma disappearance curve of radioiodinated HGH in 2 rabbits resolving into 2 single exponential equations, A and B. The rate of disappearance of curve A ($t^{1/2}$) is 8 min, whereas that of B is 70 min. It is evident that the biological half-life of HGH is markedly shortened in pregnant rabbits, as compared with the results obtained in experiments with non-pregnant rabbits. This finding was similar to that made in pregnant women^{8,9}.

No significant TCA-precipitable radioactivity could be detected in the amniotic fluid and supernatants of the homogenized foetuses. This proves that there is no transfer of GH through the placenta in rabbits during gestation. Consequently, any effect which maternal GH may have on the foetus can only be indirect.

Zusammenfassung. Die Plasma-Verschwindungskurve des präzipitierbaren TCA in schwangeren Kaninchen

zeigt nach Injektion von radiojodiertem menschlichem Wachstumshormon, dass dieses während der Schwangerschaft eine kürzere Halbwertszeit besitzt ($t^{1/2}$). Kaninchen, die 2 h nach i.v. Injektion des Wachstumshormons geopfert wurden, wiesen einen Transferrangel zu den Foeten auf.

Z. LARON, S. MANNHEIMER,
and S. GUTTMANN

Department of Pediatrics, Tel-Aviv University Medical School, Rogoff-Wellcome Medical Research Institute, Beilinson Hospital, Petah Tikva, and Isotope Department, Weizmann Institute of Science, Rehovoth (Israel), May 22, 1966.

⁹ Z. LARON, S. MANNHEIMER, and S. GUTTMANN, *Nature* 207, 298 (1965).

Permanent Culture of an Aphid on a Totally Synthetic Diet

Since an aphid, *Myzus persicae* (Sulzer), was first successfully reared through its larval stages on a synthetic diet¹ the method has been applied to other species with considerable success, notably the pea aphid, *Acyrtosiphon pisum* (Harris), which was reported to go through 3 successive generations although of progressively diminishing size^{2,3}. By contrast, *Myzus persicae* failed to produce a viable second generation; in spite of a ten- to twelvefold weight increase during first generation growth, proportionally better than that reported for *A. pisum*, second generation larvae at best sometimes doubled their weight, molted once or twice, and then, with rare exceptions, ceased growing and died⁴. Recently this impasse was broken and we now have a culture currently in its 20th successive generation on synthetic diet.

The changes which first allowed a second generation to be reared were probably twofold: the incorporation of iron in the diet (by the routine inclusion of a small amount of U.S.P. Salt Mixture No. 2, a precautionary modification, following the use of it in pea aphid diet³), coupled subsequently with more stringent care in storing diet so as to avoid pre-experimental loss of ascorbic acid⁵.

These modifications, though allowing viable second, and sometimes third generations to develop, caused little, if any, improvement in growth during the first generation, which continued, as before, to become adult at weights of about 250 μ g for *apterae* and 300 μ g for *alatae*. Last summer we tested crude nucleic acid in the diet, and were rewarded with a doubling in the weight of our first-generation adults: *apterae* of 400–500 μ g and *alatae* of 500–600 μ g – as heavy as in many plant-reared cultures. At the same time it became routinely possible to rear the third generation, but these, though living as adults for many weeks, totally failed to deposit a single fourth generation larva.

Since a mixture of appropriate nucleotides failed to substitute effectively for crude nucleic acids, it seemed probable that we were dealing with active impurities

rather than the nucleic acids themselves. That this was so became apparent when a mixture of the trace minerals iron, zinc, manganese and copper was found to be about as effective in growth as the crude nucleic acids. Since

Composition of diet

| L-amino acids (mg): | | Sucrose (g) | | 15 |
|---------------------|-----|---------------------------------------|-----------|-------------|
| Alanine | 100 | Ascorbic acid | (mg) | 100.0 |
| Arginine | 270 | Thiamin | (mg) | 2.5 |
| Asparagine | 550 | Riboflavin | (mg) | 0.5 |
| Aspartic acid | 140 | Nicotinic acid | (mg) | 10.0 |
| Cysteine HCl | 40 | Pyridoxin | (mg) | 2.5 |
| Glutamic acid | 140 | Folic acid | (mg) | 0.5 |
| Glutamine | 150 | Ca pantothenate | (mg) | 5.0 |
| Glycine | 80 | Inositol | (mg) | 50.0 |
| Histidine | 80 | Choline chloride | (mg) | 50.0 |
| Isoleucine | 80 | Biotin | (mg) | 0.1 |
| Leucine | 80 | KH ₂ PO ₄ | (mg) | 500 |
| Lysine HCl | 120 | MgCl ₂ · 6H ₂ O | (mg) | 200 |
| Methionine | 40 | | | |
| Phenylalanine | 40 | As | As | |
| Proline | 80 | seques- | elemental | |
| Serine | 80 | trene | metal | |
| Threonine | 140 | Fe ^a | 1.5 mg | 230 μ g |
| Tryptophan | 80 | Zn ^a | 0.8 mg | 112 μ g |
| Tyrosine | 40 | Mn ^a | 0.8 mg | 113 μ g |
| Valine | 80 | Cu ^a | 0.4 mg | 65 μ g |

Water: to 100 ml, adjusted to pH 7.0 with KOH. ^a Provided as metal sequestrates (Geigy Chemical Co.), the complexes with sodium EDTA.

¹ T. E. MITTLER and R. H. DADD, *Nature* 195, 404 (1962).

² J. L. AUCLAIR and J. J. CARTIER, *Science* 142, 1068 (1963).

³ J. L. AUCLAIR, *Ann. ent. Soc. Am.* 58, 855 (1965).

⁴ R. H. DADD and T. E. MITTLER, *J. Insect Physiol.* 11, 717 (1965).

⁵ R. H. DADD, D. L. KRIEGER, and T. E. MITTLER, *J. Insect Physiol.*, in press.

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 feeble 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 in-

sects 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 literature⁶, 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 generations⁷.

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

R. H. DADD and T. E. MITTLER

Division of Entomology and Acarology, University of California, Berkeley (California, USA), June 24, 1966.

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 reactions¹. 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 ellipsometer².

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 infection³.

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).