

quality of the food source. For *Acanthomyops interjectus*, this prediction now proves to be right, at least for 2 baits of different sweetness.

Since the results of the repetition were almost identical with the ones just described, we can conclude that trail-laying in *Acanthomyops interjectus* is not an all-or-none response. Each trail-layer can regulate its contribution to the trail in 2 ways, i.e., by varying the frequency of

odorous marks and by controlling the amount of pheromone fluid released per mark. The adjustment of the pheromone quantity in the trail to the quality of the food in order to regulate the number of workers that leave the nest is therefore threefold and should prove to be a highly effective mechanism to establish optimal foraging economy⁶.

Zusammenfassung. Die Menge Spurpheromon in den Duftspuren der Ameisenart *Acanthomyops interjectus* wird der Qualität des Futters angepasst. Mit zunehmender Konzentration des gebotenen Zuckerwassers erhöht sich der Prozentsatz spurenlegender Ameisen. Jedes spurenlegende Tier setzt zudem mehr Duftmarken pro Wegeinheit. Gleichzeitig nimmt auch die Menge Spursubstanzlösung zu, die pro Marke ausgeschieden wird. Da die Anzahl der von einer Duftspur angelockten Nestinsassen mit zunehmender Pheromonmenge ansteigt, wird angenommen, dass dieser dreifache Kontrollmechanismus die Zahl der ausschwärmenden Arbeiterinnen der Futterqualität entsprechend reguliert.

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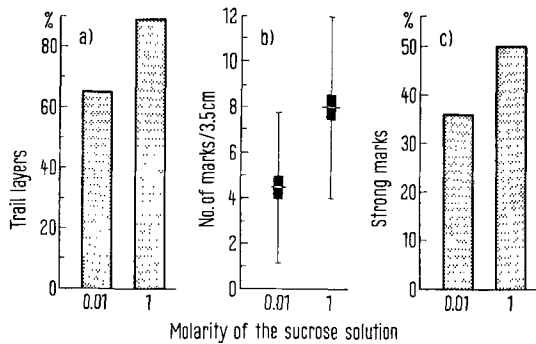


Fig. 2, a-c. The mechanisms used by *Acanthomyops interjectus* to control the amount of pheromone in trails connecting the nest with 2 food sources of different quality (0.01 and 1 M sucrose solution). (a) Adjustment by varying the percentage of trail-layers. Number of ants tested: 1 M sucrose = 75; 0.01 M sucrose = 75. (b) Adjustment by varying the number of marks set by an individual forager per 3.5 cm of its trail. Number of trails evaluated: 1 M sucrose = 67; 0.01 M sucrose = 49. Indicated are mean, standard deviation and standard error. (c) Adjustment by varying the percentage of strong marks. Total number of marks: 1 M sucrose = 536; 0.01 M sucrose = 217. The differences in all 3 cases are significant at the 0.1% level (χ^2 -test and t -test, respectively).

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Infection with Viruses of the Tacaribe Group in Thymectomized Mice

Lesions dependent on the presence of antibodies or sensitized cells may be decreased or prevented in mammals by neonatal thymectomy. This was demonstrated for lymphocytic choriomeningitis¹, and Argentine hemorrhagic fever (Junin virus)². In this preliminary work we report that the characteristic survival of thymectomized mice infected with Junin virus is equally characteristic of thymectomized mice infected with other members of the Tacaribe group of virus^{3,4}, with one exception.

Rockland mice, a strain in which wasting disease following thymectomy is delayed⁵, were thymectomized⁶ within 24 h of birth and inoculated intracerebrally within 6 h with 0.02 ml of mouse brain homogenate containing 1000 LD₅₀ doses of one of the following members of the Tacaribe group of virus^{3,4}: Junin (RC strain), Machupo (Carvalho strain), Tacaribe (TRVL 11573 strain), Pichinde (provided by Dr. C. SAN MARTIN⁴, who isolated this virus in Colombia from *Oryzomys abbigrularis*), and Amapari (L17.15B strain). Nonthymectomized new-born Rockland mice of the same age were injected intracerebrally with similar amount of virus. All mice were examined daily for neurological symptoms characteristic for these viral infections². Thymectomized mice which died spontaneously or when the period of observation was complete were examined macroscopically and microscopically to determine whether thymectomy was complete. Mice with remains of thymus were rejected from the results. Almost all thymectomized mice infected with Junin, Machupo,

Tacaribe, or Pichinde virus survived (98%, 100%, 97% and 96%, respectively); all control mice died between the 5th and 21st day after infection (Table) and had neurological symptoms typical of virus infection². In contrast, neither thymectomized nor nonthymectomized mice infected with Amapari virus survived longer than 20 days.

We reported that Junin virus could be recovered from the brains of surviving thymectomized mice 50 days after infection. Serum antibodies were never detected in these mice; spleen from immune, but not normal mice inhibited the effect of thymectomy but immune serum did not⁵. These findings demonstrate the importance of delayed hypersensitivity in the pathogenesis of infections with Junin virus, and strongly suggest the same mechanism in infections with viruses of the same group. In this study

¹ R. H. LEVEY, N. TRAININ, L. W. LAW, P. H. BLACK and W. P. ROWE, *Science* 142, 483 (1963).

² G. A. SCHMUNIS, M. C. WEISSENBAKER and A. S. PARODI, *Arch. ges. Virusforsch.* 21, 201 (1967).

³ R. M. TAYLOR, *Catalogue of Arthropod-Borne Viruses of the World* (Public Health Service publication 1760, USA 1968).

⁴ H. TRAPIDO and C. SAN MARTIN, in preparation.

⁵ M. C. WEISSENBAKER, G. A. SCHMUNIS and A. S. PARODI, *Arch. ges. Virusforsch.* 26, 63 (1969).

⁶ W. DISCHLER and C. RUDALI, *Revue fr. Étud. clin. biol.* 6, 88 (1961).

tolerance was artificially induced, but it is possible that in selected species tolerance is induced by intrauterine infection, providing in this manner a reservoir to maintain this type of Arbor virus in nature. The recent demonstration of immune tolerance in the cricetine rodent *Calomys callosus* infected with Machupo virus⁷ supports this hypothesis.

The results of infections with Amapari virus could be explained by the clonal theory of antibody formation⁸. On this basis we may consider that a different clone of cells responds to each virus. These clones may be asynchronous in maturation and some are not modified by thymectomy. Moreover, thymus-independent cells capable of producing immunoglobulins have been demonstrated⁹. According to this hypothesis, elimination of an immunocompetent thymus-independent clone before birth would allow the development of tolerance to Amapari virus.

Survival of thymectomized or nonthymectomized mice infected with viruses of the Tacaribe group

| Virus | No. of mice | Treatment | % of surviving mice 40 days after infection |
|----------|-------------|---------------|---|
| Junin | 60 | None | 0 |
| | 40 | Thymectomized | 98 |
| Machupo | 20 | None | 0 |
| | 20 | Thymectomized | 100 |
| Tacaribe | 35 | None | 0 |
| | 40 | Thymectomized | 97 |
| Pichinde | 27 | None | 0 |
| | 24 | Thymectomized | 96 |
| Amapari | 25 | None | 0 |
| | 30 | Thymectomized | 0 |

Differences between Amapari virus and the others tested in thymectomized mice suggest a method for testing new members of the Tacaribe group of viruses which would provide a biological basis for a division of the group. These studies could also provide insight into pathogenesis of the disease produced by these viruses in particular, and immune tolerance in general¹⁰.

Resumen. La timectomia en ratones recién nacidos los protege contra dosis mortales de virus Junin, Machupo, Tacaribe y Pichinde, pero no contra una dosis similar de virus Amapari. Todos estos virus pertenecen al grupo Tacaribe. Se discute el origen del fenómeno y la importancia del mismo.

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⁷ G. JUSTINES and K. M. JOHNSON, *Nature* 222, 1090 (1969).

⁸ M. BURNET, *The Clonal Selection of Acquired Immunity* (University Press, Cambridge 1959).

⁹ M. L. TYAN and L. A. HERZENBERG, *Proc. Soc. exp. Biol. Med.* 128, 952 (1968).

¹⁰ We express our appreciation to Dr. C. COTO (Cat. Microbiol. Parasitol., Univ. Buenos Aires), for her valuable assistance in carrying out this study, and to Drs. G. PACHECO and R. HERMAN (N.I.A.I.D., N.I.H.) for the editorial assistance.

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Peanut Hydrolysate as a Growth Medium for *Vibrio cholerae* (Ogawa)

Casein hydrolysate is a well-known and commonly used medium for the cultivation of cholera organisms and also for vaccine production at this Institute¹. A search, however, was continued to locate a suitable substitute for casein hydrolysate, since casein is costly and indigenously unavailable. There is, however, an ample amount of groundnut cake available after the removal of oil and it is commonly used as a cattle feed. Preliminary analysis of this cake showed 50.9% protein, 8.6% fat, 4.6% moisture, 2.5% ash and 33.4% carbohydrate (by difference). Phosphorus was 0.675%. It was, therefore, thought worthwhile to try the hydrolysate of this groundnut cake (containing more than 50% protein) as a growth medium. The hydrolysate was prepared using 100 g groundnut cakes plus 300 ml concentrated hydrochloric acid and refluxed for 18 h. After removal of excess hydrochloric acid and charcoal treatment, the pH was adjusted to 7.4. The nitrogen was determined by the micro Kjeldahl-method and was finally adjusted to 150 mg/100 ml. The *Vibrio cholerae* (Ogawa) culture was obtained from the Bacteriology Department of the Haffkine Institute. 10 ml of casein hydrolysate (150 mg/100 ml N) or peanut hydrolysate (150 mg/100 ml N) were distributed in each of 250 ml Erlenmeyer flasks and were inoculated with 0.5 ml of 18 h

old broth cultures of *V. cholerae* (Ogawa). The cultures were shaken for 24 h on a rotary shaker (100 rpm) at 37 °C. Another batch was also grown without shaking for the comparison of growth in casein hydrolysate and peanut hydrolysate in a stationary condition. At least 6 flasks were included in each batch and there was negligible

| Growth condition | Medium | |
|------------------|--------------------|--------------------|
| | Casein hydrolysate | Peanut hydrolysate |
| Stationary | 0.1308 | 0.4202 |
| Shaking | 0.5850 | 0.8539 |

Optical density measured of 540 μ on spectronic -20-

¹ S. S. SORHEV and M. K. HABBU, *Bull. Wld. Hlth. Org.* 3, 33 (1950).

² The authors wish to acknowledge with thanks the grant of the Diamond Jubilee Research Fellowship to M. J. M. Thanks are also due to Mr. V. P. SHINDE for assistance in the preparation of the hydrolysates and to the Director of the Haffkine Institute for his interest and encouragement in this work.