1 shows the results of analyses on 321 normal serums from different populations along with the standard phenotypes for the We and Vi subgroups. The striking feature of these data is that all the Gm(f−) individuals among Caucasian, Negro, Japanese, and Chinese groups were also Gm(n−). The only exceptions in this relationship were in the case of four serums from Asian Indians. These were rechecked both for n and Gm(f), and the results were confirmed. Among Gm(f+) Caucasians approximately 21 percent were Gm(n−); but among the Chinese there was complete correlation with Gm(f).

Although at least 20 different genetic factors have been delineated in the human γ-globulin system (*9*), these have all been determined by agglutination-inhibition reactions which involved the use of special anti-Rh serums. This system has had a number of limitations, particularly concerning quantitation. Previous efforts to obtain precipitation systems, particularly with human isoantiserums as well as rheumatoid arthritis serums, have been unsuccessful, possibly because most of the antibodies have been of the 19*S* type and the antiserums have been relatively weak. Recently rabbit antiserums have been successfuly employed in precipitating systems for genetic antigens of the lipoproteins and α_2-macroglobulins (*10*). Such heteroantiserums have also furnished antibodies which detect many of the genetic antigens of γ-globulin by the agglutination-inhibition system (*11*). Most of these antiserums, absorbed with serums negative for the specific genetic factor, do not show precipitin lines. Even so, in certain instances after partial absorption, a differentiation of normal serums on a genetic basis could be obtained. In most instances this differentiation only involved the intensity of the precipitin band, and spurs could not be observed. Such was the case for two genetic factors, Gm(b) and Gm(g), and for the early bleedings of antiserum M Ne which determined Gm(n). However, with persistent immunization this antiserum showed distinct spurs, and Gm(n+) serums showed good lines after complete absorption with Gm(n−) serums. The genetic differentiation, however, could be made with the early antiserums, despite the fact that, in these instances, such absorption removed all precipitin lines.

With the delineation of Gm(n), a genetic antigen has become available in the Ne subgroup of γ-globulin which previously was devoid of genetic factors. Previous studies of the We and Vi subgroups, where a number of genetic antigens are available, have revealed interesting differences in various populations between these subgroup antigens. This has also proved true of Gm(n) in its relation to the genetic antigens of the other subgroups. Of the known genetic factors it most closely paralleled Gm(f) in different populations. A marked exception occurred among Caucasians, where Gm(f+) individuals were Gm(n−) as well as Gm(n+). These three major gene complexes were found in Caucasians for the Vi, Ne, and We subgroups, respectively: $Gm^b\ Gm^n\ Gm^{fy}$, $Gm^b\ Gm^n-Gm^{fy}$, and $Gm^g\ Gm^n-\ Gm^{za}$. In Negroes the major gene complex was $Gm^b\ Gm^n-\ Gm^{za}$ which resembles the third Caucasian gene complex except that Gm^g is replaced by Gm^b. One explanation of this difference is that it arose through a crossover event involving the Gm^b and Gm^g genes. The continued association of Gm^n- with Gm^{za} in gene complexes of both racial groups suggests the possibility that the genes involving the Ne and We subgroups remained as a unit, and therefore they may be adjacent. The exact relative position of the genes for the Vi subgroup remains uncertain. Previous studies (*12*) have demonstrated the unusual gene Gm^{fya} in Mongoloid populations, which may have arisen through an intragenic crossover. If this were the case, and in view of the Gm^b and Gm^g relationships mentioned above, the order of the genes might be Vi, Ne, and We. Other findings, including the variation in Gm(n) in Caucasians, do not fit so readily into such a pattern, and further studies are indicated, particularly with regard to distinguishing between the variations stemming from crossover possibilities and those arising through point mutations.

H. G. KUNKEL
W. J. YOUNT
S. D. LITWIN
Rockefeller University, New York

References and Notes

1. W. D. Terry and J. L. Fahey, *Science* **146**, 400 (1964).
2. H. M. Grey and H. G. Kunkel, *J. Exp. Med.* **120**, 253 (1964).
3. H. G. Kunkel, H. M. Grey, A. Solomon, *Immunopathology*, IVth International Symposium, Monte Carlo (Schwabe, Basel, 1965), p. 220; W. D. Terry, *Proc. Soc. Exp. Biol. Med.* **117**, 901 (1964).
4. H. G. Kunkel, J. C. Allen, H. M. Grey, L. Märtensson, R. Grubb, *Nature* **203**, 413 (1964); W. D. Terry, J. L. Fahey, A. G. Steinberg, *J. Exp. Med.* **122**, 1087 (1965).
5. S. D. Litwin and H. G. Kunkel, *Nature* **210**, 866 (1966).
6. J. B. Natvig, *Nature*, in press.
7. J. B. Fleischman, R. H. Pain, R. R. Porter, *Arch. Biochem. Biophys. Suppl.* **1**, 174 (1962).
8. W. J. Yount and H. G. Kunkel, in preparation.
9. A. G. Steinberg, *Prog. Med. Genet.* **2**, 1 (1962).
10. K. Berg and A. G. Bearn, *J. Exp. Med.* **123**, 379 (1966).
11. S. D. Litwin and H. G. Kunkel, *Transfusion* **6**, 140 (1966).
12. L. Märtensson and H. G. Kunkel, *J. Exp. Med.* **122**, 799 (1965).
13. Supported by PHS grant FR-00102.

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Lethal Effects of Synthetic Juvenile Hormone on Larvae of the Yellow Fever Mosquito, Aedes aegypti

Abstract. *Emergence of adult mosquitoes is blocked after the addition of 1 part of crude synthetic juvenile hormone to 100,000 parts water. Development is arrested at stages ranging from pupae to fully formed pharate adults incapable of escaping from the pupal exuvium. Fourth-stage larvae just prior to metamorphosis are most sensitive: 40 percent were killed after being exposed for 1 day to 1 part juvenile hormone in 2 million parts water. The active material also inhibits the hatching of mosquito eggs.*

Ten years ago, the promise of juvenile hormone as an insecticide was evident in tests performed on the first hormonally active extracts prepared from male *cecropia* moths. When applied topically to silkworm pupae, the hormone penetrated the unbroken integument and caused lethal derangements of metamorphosis (*1*). Subsequently, these findings have been confirmed in tests of the authentic juvenile hormone of the *cecropia* silkworm, as well as of naturally occurring analogs and synthetic materials showing juvenile hormone activity (*2*).

The most active synthetic material available appears to be a product formed by treatment of ethanolic solutions of farnesoic acid with hydrogen chloride (*3*). This crude synthetic material shows a high degree of juvenile hormone activity when tested on immature insects ranging from the most primitive wingless Thysanura to the most highly evolved Hymenoptera (*4*). We now describe the action of this

Table 1. Effects of crude synthetic juvenile hormone on the metamorphosis of *Aedes aegypti*. For the control, solvent alone was applied to the mature fourth stage larvae.

Stage	Treated animals	
	Number	Emerging adults (%)
Early 4th stage larvae*	75	72
Middle 4th stage larvae†	95	93
Late 4th stage larvae‡	49	31
Mature 4th stage larvae§	99	0
Pupae	12	100
Control	12	100

* Adult eyes invisible. † Eyes linear. ‡ Eyes crescentic. § Eyes with truncate apex; pupal air trumpets invisible.

synthetic juvenile hormone analog on the yellow fever mosquito, *Aedes aegypti* (*5*).

The analog was synthesized (*3*) and used without further purification. One percent (weight/volume) of the hormonally active oil was dissolved in acetone and stored in the refrigerator. In each experiment, a measured volume (0.05 to 0.3 ml) was stirred into the contents of glass jars containing 200 ml of distilled water and a homogeneous group of 10 to 20 mosquito larvae at specific stages in development. In control experiments the water was treated with the solvent alone or with an equivalent solution of farnesol. The temperature was maintained at 21° ± 2°C, and food (pulverized pellets of Purina rabbit chow) was added after the 1st day of treatment.

Larvae at the outset of the final (fourth) larval stage were placed in 200 ml of water containing 3 mg of the crude synthetic material; all underwent pupation, but no adult mosquitoes emerged. Metamorphosis was blocked at stages ranging from pupae to fully formed pharate adults incapable of escaping from the old pupal cuticle. In parallel experiments in which the water was renewed daily and treated with fresh hormone, about one-fourth failed to pupate and, of the remainder which pupated, only a few were able to begin adult development before their development was blocked. The same result was obtained in experiments performed on mature fourth-stage larvae.

Table 2. Effects of 1 day of exposure to crude synthetic juvenile hormone on the metamorphosis of mature fourth stage *Aedes aegypti* larvae.

Dose (mg/200 ml)	Larvae (No.)	Emerging adults (%)
0	54	96
0.05	72	93
0.1	64	59
0.5	80	13
1.0	64	5
3.0	188	0

In control experiments in which larvae were exposed either to the solvent alone or to an equivalent solution of farnesol, virtually all individuals underwent normal metamorphosis.

The effects of the synthetic hormone were studied in further detail. Homogeneous groups of larvae were exposed for 1 day to 3 mg of crude synthetic material in 200 ml of water and then transferred to distilled water. Results show that mature fourth-stage larvae just prior to metamorphosis are most sensitive to the hormone analog (Table 1). Sensitivity at this stage was calibrated by exposure to graded doses for 1 day. Forty percent were killed by about 1 part of the crude synthetic material in 2 million parts of water (Table 2).

When dispersed in water, the material was fully effective for at least 1 day. However, by the end of 1 week, the dispersion became relatively ineffective, presumably because of breakdown of the hormone analog by bacteria and other agents.

Males which survived and emerged as adults in the presence of low doses of hormone were affected in a surprising way. After prior exposure to hormone, many were unable to accomplish the 180-degree rotation of the genitalia which is necessary for successful reproduction. Moreover, we have confirmed the ability of the synthetic hormone to block the embryonic development of mosquito eggs, a phenomenon already reported for three other species of insects (*6*).

ANDREW SPIELMAN
Department of Tropical Public Health, Harvard School of Public Health, Boston, Massachusetts

CARROLL M. WILLIAMS
Biological Laboratories, Harvard University, Cambridge, Massachusetts

References and Notes

1. C. M. Williams, *Nature* **178**, 212 (1956).
2. K. Sláma and C. M. Williams, *Proc. Nat. Acad. Sci. U.S.* **54**, 411 (1965); *Biol. Bull.* **130**, 235 (1966); H. A. Schneiderman, A. Krishnakumaran, V. G. Kulbarni, L. Friedman, *J. Insect Physiol.* **11**, 1641 (1965).
3. J. H. Law, C. Yuan, C. M. Williams, *Proc. Nat. Acad. Sci. U.S.* **55**, 576 (1966).
4. C. M. Williams, unpublished findings.
5. In less extensive studies essentially the same results have been obtained for the mosquito *Culex pipiens*.
6. K. Sláma and C. M. Williams, *Nature* **210**, 329 (1966); L. M. Riddiford and C. M. Williams, *Proc. Nat. Acad. Sci. U.S.*, in press.
7. Supported in part by NIH training grant No. AI46 and NSF grant GB 3232. We thank V. Skaff for technical assistance.

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Multiple Sclerosis: Correlation between Immunoglobulin-G in Cerebrospinal Fluid and Brain

Abstract. *There is a positive correlation between the concentration of immunoglobulin-G in plaques of demyelination due to multiple sclerosis (as well as in white matter of normal appearance) and the concentration of this globulin in the cerebrospinal fluid. The tentative conclusion drawn from these results was that, in patients with multiple sclerosis, the increase in immunoglobulin-G in the cerebrospinal fluid is a reflection of an excess of this globulin in the brain.*

Kabat *et al.* (*1*) have reported that the percentage of total protein in the form of γG (immunoglobulin-G) was elevated in the cerebrospinal fluid of most patients with multiple sclerosis. Because the percentage of γG in the serum was normal, they suggested that the increase in the cerebrospinal fluid might be due to "diffusion" from excess γG formed within the tissues of the central nervous system. No further evidence has yet been presented to support this hypothesis (*2*), except for our earlier reports (*3*).

Approximately 20 ml of fluid was removed from the third ventricle at the time of autopsy from seven of ten multiple sclerosis patients; the cerebrospinal fluid of nine of the ten patients had been examined when the patients were still living. The fluid was cleared of cellular debris by centrifugation (900 relative centrifugal force RCF) for 15 minutes and passage through a Millipore filter (0.45 μ); storage was in a sterile airtight tube at 4°C. The γG was determined by the immunochemical method of Kabat *et al.* (*4*), and the total protein was determined by a colorimetric method—that is, biuret reagent was added to a washed trichloroacetic acid precipitate. For comparison, normal cerebrospinal fluid, obtained from 89 medical students, was processed and examined in the same way.

The mean for total protein (mg/100 ml) and γG (percentage of total protein), plus or minus the standard deviation (SD) for the spinal fluids of 89 medical students was 38 ± 10 mg per 100 ml and 9.9 ± 2.6 percent γG, respectively. For the patients the individual values for cerebrospinal fluid and ventricular fluid total protein and percent of γG are shown in Fig. 1 and Table 1.