That these 11 values (mosaic units) are no accident is indicated as follows.

1) With the exception of 21.6 cm, mosaic units lie in a distinct series, each being the sum of the preceding two (within 0.1 cm).

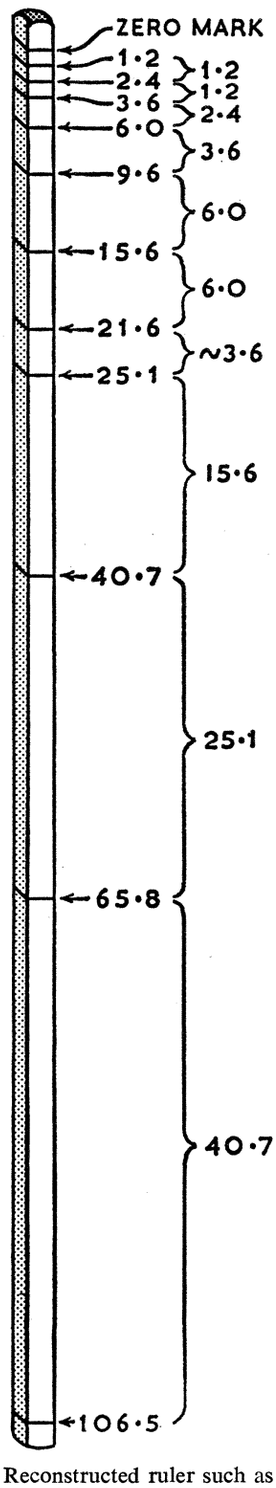

Fig. 1. Reconstructed ruler such as ancient mosaicists might have used. All lengths in centimeters. Measuring between each pair of calibrations, as opposed to measuring from the zero mark to each calibration, does not yield any extra values.

2) The typical dimensions of patterns greater than 106.5 cm have been successfully predicted (*2*) within 0.3 cm by extrapolating this series. But, substituting integers 1, 2, 3, . . . 14 for x, I find the relation

$$y = 1.197 \times \left[\frac{1}{\sqrt{5}}\left(\frac{1+\sqrt{5}}{2}\right)^x - \frac{1}{\sqrt{5}}\left(\frac{1-\sqrt{5}}{2}\right)^x\right] \text{cm}$$

yields a set of values for y which fit the observed mid-interval modal values ($c = 0.1$ cm) up to, and beyond, 106.5 cm within 0.07 cm (ignoring 21.6 cm).

3) Mosaic units (including 21.6 cm) lie within the ± 0.25 cm range of each value in a set of 11, experimentally shown (*3*) to be the most efficient pattern sizes in terms of stone packing.

4) Mosaic units (including 21.6 cm) lie within ± 0.1 cm of each of the 11 most efficient pattern sizes (*4*) predicted by a theoretical study of varied-particle packing.

I can find no likely relation between any of the lengths of 6646 different integral numbers and fractions of 89 pertinent known ancient standard units of length and the 11 mosaic units.

The only known ancient accounts of floor mosaic construction are apparently (*5*) those of Vitruvius, and of Pliny (who largely echoes Vitruvius). The Vitruvian sources state (*6*) that floor mosaics were ". . . *ad regulam . . . exacta . . . struantur . . .*" (that is, accurately formed by ruler).

Presumably this ruler was scaled in the 11 mosaic units. If it was like the ancient rulers still in existence (*7*), it would be a wooden stick of square section, with transverse cuts for calibrations. A reconstructed mosaic unit ruler is shown in Fig. 1.

I hope to draw the attention of those who possess ancient rulers to the possibility that an original mosaic unit ruler might have come down to us.

RICHARD E. M. MOORE

Anatomy Department, Guy's Hospital Medical School, London, S. E. 1.

References and Notes

1. R. E. M. Moore, *Nature* **209**, 128 (1966).
2. ———, *Bull. Inst. Classical Stud.* **11**, 87 (1964).
3. ———, *Nature* **217**, 482 (1968).
4. ———, *Intermedica* 31 (summer 1966).
5. ———, *Amer. J. Archaeol.* **72**, 57 (1968).
6. For example, British Museum Harleian MS 2767, p. 95^{vesro}.
7. For example, Roman-Egyptian cubit ruler, Petrie Collection 1935-459, Science Museum, London.

8. Supported by grants from the Worshipful Company of Goldsmiths, University College London, and the Leyerhulme Trust. I thank Prof. Roger Warwick for great encouragement.

31 July 1968

Immunological Response of Male Cockroaches to Injection of Tetrahymena pyriformis

Abstract. *After injection of living tetrahymena into the hemocoels of male cockroaches, recovered hemolymph immobilizes washed ciliates. The immobilizing material is sensitive to heat and acid, and can be separated with ammonium sulfate. Hemolymph from immune animals confers protection in another animal into which it is injected. Immobilizing activity of the hemolymph from immune animals is associated with a nonreactive, normal protein component present in hemolymph from nonimmunized insects.*

Several species of the ciliated protozoan *Tetrahymena* are natural facultative parasites in invertebrate hosts (*1*). In addition, several strains of usually free-living organisms of this genus may be experimentally introduced into various host organisms and tissues (*2*). The free-living S strain of *T. pyriformis* will live in the hemocoel of adult female American cockroaches (*Periplaneta americana*); the ciliates are easily recovered and isolated into axenic culture, and their morphological (*3*) and

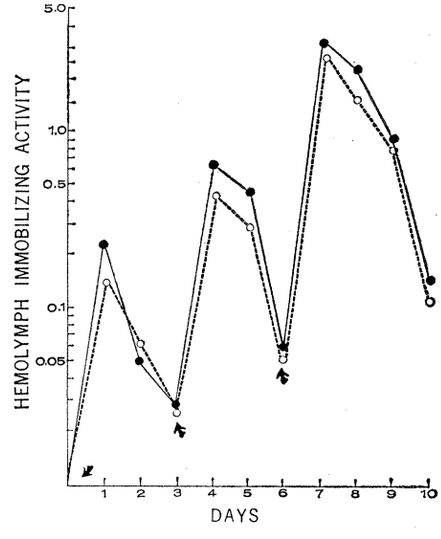

Fig. 1. Titer of immobilizing activity of cockroach hemolymph after repeated injections of *Tetrahymena pyriformis* S. Units of activity are expressed as the reciprocal of the minimum amount of hemolymph protein (micrograms) which in the standard assay effects immobilization of washed ciliates (see text). Arrows indicate time of injection of 250 μg (dry weight) of ciliates. Each assay was made in pooled diluted hemolymph from three animals. Closed circles and solid line represent data obtained upon injection of living ciliates; open circles and dotted line represent data with heat-killed ciliates.

biochemical (*4*) alterations may be studied. However, when large numbers of ciliates are injected into the hemocoels of adult male cockroaches, a large percentage of the experimental hosts dies. At a dosage that kills approximately 50 percent of the insects over a 3-day period after injection (deaths usually occurring only in the first 2 days after inoculation), ciliates cannot be recovered from the hemolymph from the survivors. This suggests that male cockroaches produce an immunological substance against the ciliates.

We now describe the immunological-like responses of male *P. Americana* to inoculation with living *T. pyriformis* S and the characteristics of a separated fraction of the hemolymph from inoculated male cockroaches.

All experiments reported were carried out as follows. The ciliates were grown in 2 percent bacteriological peptone (Nutritional Biochemicals Corp.) and 0.2 percent yeast extract (Nutritional Biochemicals Corp.) for 48 hours, and harvested by centrifugation, and washed twice in distilled water immediately before injection. Adult *Periplaneta* males were injected between the third and fourth abdominal segments with 0.1 ml of a suspension of washed tetrahymenas diluted to contain 2.5 mg (dry weight) of ciliates per milliliter. At various times after inoculation the hemolymph from living insects was collected with a Pasteur pipette after the membrane between the hind coxa and the abdomen was snipped. The pooled hemolymph from three animals (approximately 0.3 to 0.4 ml) was diluted with 1 ml of 0.5 percent NaCl. The insects were lightly anesthetized with ether during both the injection and the bleeding procedures.

Cell-free hemolymph recovered on the 3rd day after injection immobilized washed S strain ciliates grown in peptone-yeast extract. The standard immobilization test was conducted in a watch glass within a moist petri-dish chamber; to 0.2 ml of diluted and centrifuged hemolymph, 0.8 ml of washed ciliates were added; they were examined microscopically after 1 hour of incubation. Controls were cells left 1 hour in dilutions of hemolymph from uninjected male cockroaches as well as from male and female insects injected with 0.1 ml of distilled water or saline solutions (0.5 to 0.9 percent NaCl). Hemolymph from none of the controls immobilized the tetrahymenas.

Test material can be diluted with distilled water so that the minimal amounts which elicit the immobilization response can be determined. Titers of diluted hemolymph, determined by the usual twofold serial dilution procedure, are expressed as micrograms of protein per test vessel, based on analyses made on initially diluted recovered hemolymph (*5*).

In the course of multiple inoculations with either living or dead ciliates (killed, but not lysed by heating at 54°C for 10 minutes) there is increased ciliate immobilization activity (Fig. 1). Pooled diluted hemolymph from immune male cockroaches does not immobilize normal washed tetrahymenas when it is subjected to *p*H 1 for 1 hour and is then neutralized, or when it is heated at 75°C for 20 minutes. The active immobilizing material can be separated with ammonium sulfate; immobilizing activity is recovered in fraction which precipitates between 50 and 80 percent saturation.

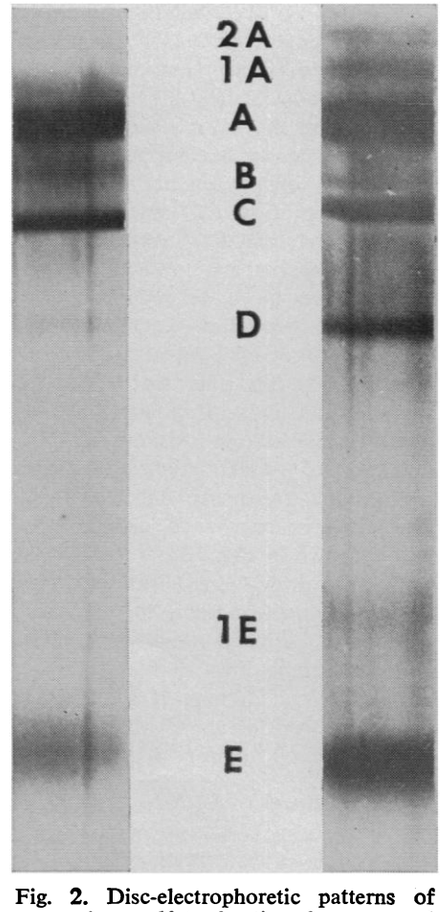

Fig. 2. Disc-electrophoretic patterns of ammonium sulfate fraction from normal (left) and immune (right) cockroach hemolymph. Migration was from the top to the bottom. Fractions were prepared from pooled samples of hemolymph from ten normal males and from ten male cockroaches immunized with three injections of 250 μg (dry weight) of ciliates each, and bled at the time of peak immunological response (Fig. 1).

Thus, the immune material elicited by injection of living tetrahymena into the male cockroaches differs from that produced by insects into which bacterial cells have been injected (*6*, *7*) in that the material produced against the ciliate appears to be protein in nature.

In common with the response elicited by bacteria in other insects (*7*, *8*), that elicited in male cockroaches by tetrahymenas is protective. After the first inoculation of ciliates, which initially results in high mortality, additional injections and higher doses can be injected with a decrease in mortality rates.

Tetrahymena can live in the hemocoel of female cockroaches. To ascertain if the immune material produced by the male can be passively transferred in a manner similar to antibacterial substances produced by other insects (*7*, *8*), we injected purified material (the 50 to 80 percent saturated ammonium sulfate fraction) into females. The immune material was prepared from pooled dilute hemolymph recovered from males given two injections of living ciliates (250 μg, dry weight, per insect); it was recovered during the peak of immobilizing activity (Fig. 1). Twenty-four hours after a single injection of 26 μg of protein of the ammonium sulfate fraction, each of ten females was challenged with a single injection of 460 μg (dry weight) of living, washed ciliates. Living tetrahymenas were recovered 48 hours after the challenge from only three females. The tetrahymenas recovered were scarce in number, and did not have the usual morphological rounding of the organisms observed upon immediate recovery of ciliates from female cockroaches (*3*); they had the normal *T. pyriformis* shape.

Disc-electrophoretic patterns were determined on the 50 to 80 percent saturated ammonium sulfate fractions obtained from normal and from immunized males to ascertain if the ciliate immobilizing material is associated with a specific protein in the hemolymph. Samples were run on 7.5 percent polyacrylamide gel at *p*H 9.5 toward the anode and at *p*H 4.3 toward the cathode at 5 ma per column. After separation, protein was detected by staining with aniline black. Only two basic proteins were detected in samples from the normal and immunized roaches; the mobilities of the two bands in both samples were identical. However, five bands of acidic proteins were separated from the fraction from normal hemolymph. Three additional bands (1A, 2A, and 1E) of acidic proteins were apparent in

the immune fraction (Fig. 2). Each of the eight bands from fractionated immune hemolymph was cut from simultaneously run unstained gels, and the protein was eluted into a small volume of dilute salt solution (*9*) by ultrasonic mixing with a microprobe. After clarification by centrifugation, material obtained from each band was assayed for immobilizing effect in the standard test system. Activity was obtained in the C band present in immune hemolymph. The C band present in normal male hemolymph does not show this activity, nor do samples in any of the three proteins present only in the fraction from immunized hemolymph. The new proteins in the immune hemolymph fraction are thus evidently formed in response to portions of the ciliate that are not associated with the immobilizing response, whereas, like the gamma globulin response in mammals, the immune material is normally present as a preformed protein fraction in the circulating fluid of the organism and becomes altered in response to immunization.

GERALD R. SEAMAN
N. L. ROBERT
Department of Biology, Roosevelt University, Chicago, Illinois 60605

References and Notes

1. J. O. Corliss, *J. Parasitol.* **50**, 111 (1960).
2. E. N. Kozloff, *J. Protozool.* **3**, 17 (1956); J. C. Thompson, Jr., *ibid.* **5**, 203 (1958).
3. G. R. Seaman and T. J. Tosney, *ibid.* **14** (suppl.) 23 (1967).
4. G. R. Seaman, in *Proceedings of the First International Congress of Parasitology*, A. Corradetti, Ed. (Pergamon Press, New York, 1966), vol. 1, p. 74.
5. O. H. Lowry, N. J. Rosenbrough, A. L. Farr, H. J. Randall, *J. Biol Chem.* **193**, 265 (1951).
6. J. D. Briggs, *J. Exp. Zool.* **138**, 155 (1958).
7. R. E. Gringrich, *J. Insect Physiol.* **10**, 161 (1964).
8. J. M. Stephens, *Can. J. Microbiol.* **5**, 203 (1959).
9. J. F. Ryley, *Biochem. J.* **52**, 483 (1952).
10. These investigations were initiated while both authors were members of the Department of Biological Sciences, Hunter College, the City University of New York; supported by NIH grants AI-106092 and AI-07830.

26 July 1968

Induction of Collagenolytic and Proteolytic Activities in Rat and Human Fibroblasts by Anti-Inflammatory Drugs

Abstract. *Confluent monolayers of either mouse or diploid human fibroblasts contained no measurable amounts of either collagenolytic (pH 5.5) or proteolytic (pH 7.5) activities. Within a few hours after exposure of these cells to anti-inflammatory drugs, significant amounts of these enzymatic activities were demonstrable extracellularly. These activities were profoundly decreased in cultures simultaneously treated with the anti-inflammatory drugs and cycloheximide or actinomycin D.*

Following the administration of corticosteroids, loss of collagen from the skin of rats is marked (*1*). A similar loss has been shown in man (*2*). In the rat, the biological half-life of insoluble cutaneous collagen is about 1 year, and the total loss of collagen in 24 hours is about 50 mg for a 200-g rat (*3*). During the day after administration of cortisol, indomethacin, and oxyphenylbutazone, but not salicylate, catabolism of collagen in the skin is more than ten times that occurring normally in the whole body (*4*). It seems unlikely that these drugs could effect such a loss by merely inhibiting the anabolism and not accelerating the catabolism of this fibrous protein.

Since collagen is located extracellularly, and since the administration of these anti-inflammatory drugs neither increases the number nor alters the kinds of cells found in the skin (*2*), catabolism of cutaneous collagen must proceed by the release of collagenolytic activity from normal skin cells into the extracellular compartment of the tissue. The skin contains enzymatic activities capable of digesting purified insoluble cutaneous collagen at *p*H 5.5 (*5*). Free collagenolytic activity and a proteolytic activity (*p*H 7.5) were found in the extracellular compartment of the skin from rats treated with steroids, neither was found in untreated control rats (*4*, *6*). Their appearance was always associated with a loss of collagen from the skin (*4*, *6*). The enzyme activities were not associated with the release of any of the well-known enzymes marking the labilization of lysosomes (*4*, *7*).

Finally, previous treatment of rats with such varied inhibitors of protein biosynthesis as puromycin [inhibiting messenger RNA (*8*)], cycloheximide [inhibiting peptide chain lengthening (*9*)], and actinomycin D [inhibiting messenger RNA synthesis (*10*)] inhibited the extracellular appearance of the collagenolytic and proteolytic activities and the loss of insoluble collagen from the skin (*11*). Previous treatment of cortisol-treated rats with 5,5-diphenylhydantoin also inhibited the appearance of collagenolytic activity and loss of insoluble collagen from the skin (*11*). Our results suggest that the induction of these enzymatic activities results from the drug-produced derepression of an operon within the mouse heteroploid or human diploid fibroblast in confluent monolayer cell culture.

Strain L fibroblasts were obtained (*12*) and grown in Eagle's essential medium containing 10 percent fetal calf serum in the usual fashion in 32-ounce pharmacy bottles until they reached confluency. The confluent monolayers of these cells (more than 20×10^6 cells per bottle) were allowed to stand for 5 days in medium to which had been added 12 μg of ascorbate per milliliter. The monolayer was then rinsed with sterile isotonic saline until no more protein could be determined (*13*) in the washings. These monolayers were then harvested, counted in a hemocytometer, and suspended in 1 ml of medium (without serum or ascorbic acid) per 4×10^6 cells. To some of these cell suspensions was added cortisol succinate, indomethacin, or oxyphenylbutazone (20 μg/ml). Some of these treated cultures also received simultaneously either cycloheximide or actinomycin D (5 μg/ml). After 0, 2, 3, 4, 5, and 6 hours, samples of medium and cells were collected together, homogenized in a Potter glass homogenizer, and assayed for proteolytic and collagenolytic activities.

Proteolytic activity was determined by incubation of denatured hemoglobin at *p*H 7.5 with 1 ml of homogenate for 16 hours at 36°C (*14*). The amount of protein soluble in trichloroacetic acid released under these conditions was expressed in micrograms of tyrosine equivalents per 4×10^6 cells (*4*, *14*).

Collagenolytic activity was determined by incubation of 1 ml of the homogenate with 10 mg of purified, native, insoluble rat-skin collagen at *p*H 5.5 for 16 hours at 24°C (*4*, *6*). The amount of peptide-bound hydroxyproline rendered soluble under these conditions was determined after hydrolysis in 4*N* HCl at 100°C for 8½ hours (*15*). The results were expressed in micrograms of hydroxyproline released per 4×10^6 cells.

The insoluble cutaneous collagen substrate was prepared as described previously (*4*–*6*) and contained less than 2 percent noncollagenous nitrogen.