light, a system of monochromatic light and dark fringes were focused in the whole field of view.

The cricket spermatids were mounted in an isotonic insect saline medium for interferometric investigations. The phase-change retardation of the nebenkern becomes visible as the lateral shift of the fringes in the image which was photomicrographed (Figure). The accuracy obtained by this method is expected to be $\pm$ 0.005 wavelength. The average optical path difference determined from the photomicrographs is 0.58 wavelength of the green light; thus the absolute optical path difference of the nebenkern is (0.58×0.546 μ). Cell diameters were measured with a micrometer ocular.

The basic formula relating the optical path difference to the dry mass of the mitochondrial nebenkern is the same as used in refractometry. However, since in this case the cells were suspended in isotonic saline medium, a correction factor is added to the formula:

$$M = \frac{\Phi A}{100\alpha} + (\mu_m - \mu_w)\frac{At}{100\alpha}$$

where μ_m, μ_w are the refractive indices of the saline medium and water, respectively. t is thickness equivalent to the diameter.

The dry weight mass of the nebenkern is determined to be 12.56×10^{-12} g%.

The value 11.24×10^{-12} g obtained for total dry mass of mitochondrial nebenkern by refractometry agrees well with the dry mass value of 12.56×10^{-12} g obtained by interferometry. Differences in values were well within the errors of both methods[10-12].

Résumé. L'auteur a effectué des mesures réfractométriques et interférométriques sur le résidu sec, concentré, de la matière mitochondriale du «nebenkern» des spermatides du Grillon. La valeur obtenue par réfractométrie (11×10^{-12} g) en utilisant le microscope à interférence de A. O. Baker concorde avec celle qui a été obtenue par interférométrie (12.56×10^{-12} g) avec le nouveau microscope à interférence de Leitz.

H. C. Chopra[13]

Division of Cell Biology, Institute for Muscle Disease, Inc., New York (U.S.A.), June 1, 1962.

[10] H. G. Davies, *General Cytochemical Methods* (J. F. Danielli, Ed., Academic Press Inc., New York 1958), vol. 1, p. 57.

[11] K. F. A. Ross, *General Cytochemical Methods* (J. F. Danielli, Ed., Academic Press Inc., New York 1961), vol. 2.

[12] The author wishes to thank Dr. W. G. B. Casselman for teaching interference microscopy. This work was supported by a fellowship grant from the Muscular Dystrophy Association of America.

[13] Present address: The Rockefeller Institute, New York (U.S.A.).

The Perception of Vibration by the Subgenual Organ in *Zootermopsis angusticollis* Emerson and *Periplaneta americana* L.

In their investigations of the vibration-sensitivity of the insect subgenual organ, Autrum[1,2], and Autrum and Schneider[3] have concluded that there was a transformation of the stimulus (*Reiztransformation*), in which the vibration gave rise to vortices in the blood around the subgenual organ. The constant pressure of these vortices was held to be responsible for stimulating the sensory cells. During the course of investigations of the vibration-sensitivity of *Z. angusticollis* and also of *P. americana*, evidence has accumulated which suggests a slightly different interpretation.

In both insects oscillographic recordings were taken with electrodes in the ventral nerve cord, but in the cockroach mainly with electrodes in the femur of an isolated leg. The legs were rested on a surface which could be vibrated by the coil of a suitably modified moving-coil loudspeaker worked from an audiofrequency oscillator.

In both insects the response to a pulse of vibration had a latency of 10–20 msec and died out after about the first half second (Figure 1 for the cockroach preparation). However, a series of rapid pulses at the same frequency of vibration elicited a synchronous non-adapting response for up to at least 30 pulses/sec for *P. americana* (Figure 1). At between 5 and 20 pulses/sec for *Z. angusticollis*, the response, although synchronous, tended to be lost after about 10–30 pulses (Figure 2). After a break of 1/50 sec in a long pulse of vibration, an immediate response can be recorded, and a phenomenon frequently encountered with a cockroach preparation is a response originating from the end of the pulse as well as from the start of the next (Figure 1). Further, after the response to a long pulse has died away, a synchronous response can be obtained to modulation of the vibration by tapping the support with a glass rod (Figure 3).

This evidence clearly suggests that the sense-organ is responding not to steady-state conditions, but to phenomena that precede or interfere with the steady state. Before going further it is necessary to consider the structure of the subgenual organ which has been studied in *Z. angusticollis*. (Further details of this will be published elsewhere.)

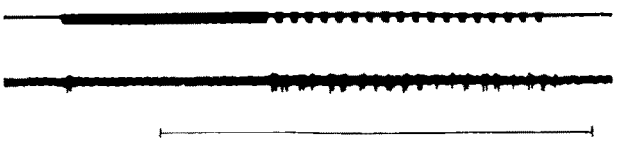

Fig. 1. Responses recorded from the cockroach femur to vibration with a frequency of 1000 c.p.s., partly in pulses of 30/sec.

Fig. 2. Responses recorded from the nerve cord of the termite to vibration with a frequency of 1250 c.p.s., in pulses of 10/sec.

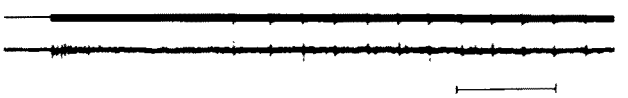

Fig. 3. Responses recorded from the nerve cord of the termite to vibration with a frequency of 1250 c.p.s., modulated by taps with a glass rod.
Scale line = 1 sec in each case.

[1] H. Autrum, Naturwiss. *30*, 69 (1942).

[2] H. Autrum, in *Handbook of Physiology*, Section 1 (Ed. Field, Washington 1959), vol. 1.

[3] H. Autrum and W. Schneider, Z. vgl. Physiol. *31*, 77 (1948).

The proximal scolopale cells lie in the blood-space of the tibia and are attached at their distal extremities to the accessory cells. The latter are closely bound together by a network of tendrils, presumably collagenous in nature, which come together in a broad stem to attach to the cuticle.

Depending on the conditions of damping, the sudden application of a force to a mass will initiate a harmonic free vibration of that mass. The displacement, x, is given by an equation of the form

$$x = Ae^{-\delta t} (\cos \omega_r t - \gamma)$$

Where ω_r is the angular velocity at the natural frequency, and A and γ are constants proportional to the applied force. Under normal conditions this vibration will soon be damped out. If the applied force is itself harmonic, as in the cases considered here, it follows there will be an initial transient vibration set up. This will revert to the vibration due to the impressed force as soon as the free vibration has been damped out. The displacement will then be given by an equation of the form

$$x = X \cos \omega t$$

where X is the maximum displacement, and ω is the angular velocity of the impressed force. Therefore the initial effect of the forced vibration of the leg will be to set the cells of the subgenual organ into free (transient) vibration, but since the distal cells are bound to each other and to the cuticle, their natural frequency will be different from that of the proximal cells. Hence while the transient vibrations last, there will be a rapid and complex variation of forces at the junctions of the proximal and distal cells, which could cause the nervous discharge. If this is so, any sufficiently abrupt displacement from equilibrium or steady-state conditions must be expected to initiate nervous discharge, which is clearly in accord with the results obtained.

Zusammenfassung. Aus den Ergebnissen einer elektrophysiologischen Untersuchung des Subgenualorgans zweier Insekten lässt sich ableiten, dass eine Erregung der Sensillen nur nach einer plötzlichen Störung des Beines aus der Ruhe oder dem Gleichgewichtszustand auftritt.

Es wird angenommen, dass eine plötzliche Verlagerung der Stiftzellen und der distalen akzessorischen Zellen des Subgenualorgans aus der Ruhe oder dem Gleichgewichtszustand sie während einer sehr kurzen Periode in Eigenschwingung versetzt. In Abhängigkeit von ihrem Bau dürfte die Frequenz der Eigenschwingung bei jeder der zwei Zellengruppen verschieden sein. Infolgedessen wird es, bis zur Einstellung eines neuen Gleichgewichtszustandes, zu einer schnellen Veränderung der Kräfte an der Verbindung zwischen Stiftzellen und akzessorischen Zellen kommen, was Nervenimpulse auslösen könnte.

P. E. HOWSE

Department of Zoology, Queen Mary College, University of London (England), June 27, 1962.

An Alkali Resistant Factor with B_{12} Activity for Protozoa and Man[1]

Our studies on the effect of flushing doses of cyanocobalamin (vitamin B_{12}) in dogs and man revealed that B_{12} displaced not only hepatic bound B_{12} but also an alkali-resistant thermostable factor (ARF) which supported the growth of the B_{12}-requiring microorganisms *Lactobacillus leichmannii, Escherichia coli* 113-3, *Euglena gracilis* and *Ochromonas malhamensis*[2]. Growth could not be attributed to deoxyribosides[3–5] since the latter two organisms do not respond to nucleic acid derivatives[6]. Also, ARF failed to support growth of a thymine-thymidine-requiring *E. coli* mutant. Unlike B_{12} or related cobamides, ARF is stable at 118°–121°C and pH 11.5–12 for 30–60 min without added reducing agents[7,8]. ARF was not detected in the circulation of man unless displaced by B_{12}. It was found in human and beef liver and also in alkali extracts from the culture medium of a thermophilic bacillus grown at 55°C. The present communication describes methods of preparation and some of the chromatographic, microbiological, and clinical properties of ARF.

Materials and Methods. Source of ARF. (a) Human: Hepatic venous blood was obtained from 12 normal subjects and 8 patients with cirrhosis 5 min to 2 h after intravenous administration of 100 µg of B_{12}[9]. Surgical liver biopsies were obtained from 8 normal subjects and percutaneous biopsies[10] from 2 patients with pernicious anemia.

(b) Animal: Available commercial beef liver powders were analyzed for their ARF content. The best source was found to be liver extract concentrate 1:20 obtained from Nutritional Biochemicals Corp., Cleveland (Ohio).

(c) Microbial: *Bacillus coagulans* ATCC 12990, a thermophile produced ARF. It was grown at 55°C in a medium consisting of corn-steep liquor (5.0 ml), NaCN (2.0 mg), cobalt (4.0 · mg as $CoSO_4 \cdot 7H_2O$), citric acid · H_2O (100 mg), triethanolamine (300 mg), distilled water (to 100 ml) adjusted to pH 6.1 with KOH[11].

Cobalt was not essential for ARF production, the same concentration of iron, added as $FeSO_4 \cdot 7H_2O$ could be substituted. Although ARF activity appeared without these ions, either ion stimulated ARF production by the bacillus. The medium was inoculated with an homogenate from a loopful of *B. coagulans* grown on nutrient agar overnight. Maintenance and growth of these thermophiles have been described[12].

ARF assay. B_{12} and ARF were assayed with (a) *L. leichmannii* ATCC 7830, (b) the mutant *E. coli* 113-3, (c) *E.*

[1] These studies were supported by NIH Grants A-4437 (C1); C-4956 (C2); H-4530 (C2); and Clinical Research Center Grant OG-17R1; and a Grant from the National Vitamin Foundation.

[2] C. M. LEEVY, H. BAKER, O. FRANK, and H. ZIFFER, J. clin. Invest. *40*, 1057 (1961).

[3] T. PETTERSON, G. OSTLING, and R. GORDIN, Acta med. scand. *148*, 219 (1954).

[4] G. H. SPRAY and K. B. TAYLOR, Nature *182*, 1309 (1958).

[5] M. A. BOOTH and G. H. SPRAY, Nature *193*, 74 (1962).

[6] J. E. FORD and S. H. HUTNER, Vitamins and Hormones *13*, 101 (1955).

[7] K. L. SMITH, in *Vitamin B_{12} and Intrinsic Factor*, vol. 1, 1 (1957).

[8] A. W. JOHNSON and A. TODD, Vitamins and Hormones *15*, 1 (1957).

[9] C. M. LEEVY and M. L. GLIEDMAN, New Eng. J. Med. *258*, 696 (1958).

[10] C. M. LEEVY, *Practical Diagnosis and Treatment of Liver Disease* (P. B. Hoeber, New York 1957).

[11] H. BAKER and H. ROTH, *International Hormones*, December 1959, U.S. patent 2917436.

[12] H. BAKER, O. FRANK, I. PASHER, B. BLACK, S. H. HUTNER, and H. SOBOTKA, Canadian J. Microbiol. *6*, 557 (1960).