by Dr. W. C. Levengood of the University of Michigan (*5*). Kanamycin is very effective against *Bacillus* spp. and most Gram-negative bacteria, such as *Pseudomonas aeruginosa, Serratia marcescens*, and *Proteus* spp. (*6*). These microorganisms and *Streptococcus* spp. are the main potential pathogens present in the silkworm intestine (*2, 3, 7*). Therefore, it seemed possible that kanamycin combined with aureomycin, which is effective against *Streptococcus* (*8*), might prevent disease in cecropia larvae feeding on leaves.

To test this possibility, 2.8 g of aureomycin (chlortetracycline hydrochloride) (*9*) and 0.155 g of kanamycin sulfate (*10*) were dissolved in a liter of distilled water and sprayed onto freshly cut and washed leaves of wild cherry (*Prunus virginiana*). The leaves were air-dried and then fed to cecropia larvae kept under normal laboratory conditions.

Of 54 newly hatched larvae, 36 survived to pupation. All animals were remarkably healthy, and all losses were attributable to accidents, mainly in the first instar. No deaths occurred in the fourth or fifth instar.

The mixture of aureomycin and kanamycin permits, for the first time, experiments with cecropia under laboratory conditions. After certain types of experimentation, a few larvae may succumb to the disease. But this antibiotic mixture prevents the usual pandemic, even within the same group. These antibiotics also permit the rearing of *Antheraea polyphemus* and *Antheraea pernyi* in the laboratory, and it seems likely that infections of other insects reared in the laboratory can be controlled in this same manner.

LYNN M. RIDDIFORD
Biological Laboratories, Harvard University, Cambridge, Massachusetts

References and Notes

1. G. B. Staal, personal communication.
2. G. Benz, in *Insect Pathology*, E. A. Steinhaus, Ed. (Academic Press, New York, 1963), vol. 1, p. 299; E. A. Steinhaus, *Proc. Int. Congr. Entomol. 10th* **4**, 725 (1958); ———, *Ecology* **39**, 503 (1959); ——— and J. P. Dineen, *J. Insect Pathol.* **2**, 55 (1960).
3. E. G. Afrikian, *J. Insect Pathol.* **2**, 299 (1960).
4. C. M. Williams, personal communication.
5. W. C. Levengood, personal communication.
6. A. Gourevitch, V. Z. Rossomano, T. A. Puglisi, J. M. Tynda, J. Lein, *Ann. N.Y. Acad. Sci.* **76**, 31 (1958).
7. O. Lysenko, *J. Gen. Microbiol.* **18**, 774 (1958); W. A. Smirnoff, *Can. J. Microbiol.* **11**, 703 (1965).
8. Lederle Laboratories, Pearl River, N.Y., *Circular 47594-UX48* (1966).
9. Gift of Lederle Laboratories.
10. Gift of Bristol Laboratories.

13 July 1967

Protein Components in the 40S Ribonucleoprotein Particles in Escherichia coli

Abstract. *The 40S ribonucleoprotein particle in* Escherichia coli *cells, accumulated in the presence of a low concentration of chloramphenicol, lacks at least four ribosomal structural protein components which are present in the mature 50S ribosomal subunit. The 40S ribonucleoprotein prepared by exposing the 50S ribosomal subunit to a concentrated lithium chloride solution may also be deficient in the same protein components.*

It has been proposed that the 40*S* to 43*S* ribonucleoprotein particle (RNP) (hereafter referred to as the 40*S* precursor RNP) found in bacterial cells is a precursor in the formation of the 50*S* ribosomal subunit. The 40*S* precursor RNP can be detected (i) by pulse-labeling of *Escherichia coli* cells with isotopic precursors of RNA or protein (*1*); (ii) during the transformation of experimentally accumulated early intermediates to the ribosomal subunits (*2*); or (iii) in a retarded formation of the 50*S* subunit in a mutant strain (*3*). An aspect of ribosome formation may be expressed as a progressive attachment of a series of ribosomal proteins, probably in a specific order, to the nascent 16*S* and 23*S* ribosomal RNA's (rRNA) to complete the 30*S* and 50*S* ribosomal subunits, respectively (*4*). Thus, it may be expected that the 40*S* precursor RNP lacks certain definite components of the structural proteins which are present in the 50*S* subunit.

We have observed that when *E. coli* cells were treated with a low concentration (0.6 to 1.0 μg per milliliter of culture medium) of chloramphenicol, a considerable amount of the 40*S* RNP and other RNP's having various *S* values accumulated (*5*). The 40*S*

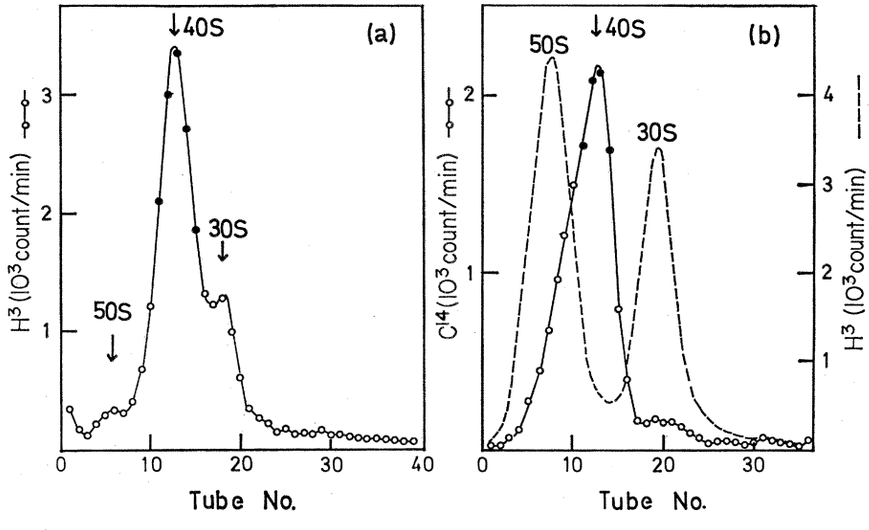

Fig. 1. Sucrose density-gradient centrifugations of the 40*S* precursor RNP and physically produced 40*S* RNP. (a) Sedimentation profile of H^3-lysine-labeled 40*S* region taken after the first sucrose density-gradient centrifugation. *Escherichia coli* cells were treated for 20 minutes with 0.7 μg of chloramphenicol per milliliter in the presence of 200 μc of H^3-lysine. A cell extract made with a mixture of $10^{-4}M$ Mg^{++} and $10^{-2}M$ tris-HCl (*p*H 7.4) containing 5 μg of deoxyribonuclease per milliliter was centrifuged on a sucrose density gradient (5 to 20 percent) at 21,000 rev/min for 16 hours and fractionated. The material from the 40*S* region was dialyzed against the described buffer for 5 hours, and again centrifuged on a sucrose density gradient. (b) Sedimentation profile of the 40*S* particles obtained by a LiCl treatment of the 50*S* ribosomal subunit. Cells of *E. coli* were labeled with C^{14}-lysine for three generations. The labeled 50*S* ribosomal subunit was obtained by a sucrose density-gradient centrifugation. The preparation was then mixed with about 6 mg of 70*S* ribosomes, treated with 1.25*M* LiCl containing $10^{-4}M$ Mg^{++} and $2.5 \times 10^{-2}M$ tris-HCl at *p*H 7.4 in the cold, and centrifuged at 45,000 rev/min for 6.5 hours to remove proteins which had been released from the labeled 50*S* subunit. The pellet was homogenized and centrifuged on a sucrose density gradient (–o–o–). The 50*S* and 30*S* ribosomal subunits labeled with H^3-uridine were used as references (– – – –). This final centrifugation was at 21,000 rev/min for 16 hours at 4°C for (a) and (b). The fractions indicated by the black circles in the figures were used for the analyses of protein components.

RNP, which has been found to contain an incompletely methylated nascent 23*S* rRNA, was transferred to the 50*S* ribosomal subunit upon removal of chloramphenicol from the growth medium, and, at the same time, addition of 5 μg of actinomycin D per milliliter to stop further synthesis of rRNA (*6*). Thus, the 40*S* RNP that accumulated in the presence of a low concentration of chloramphenicol may be considered a precursor of the 50*S* ribosomal subunit, but not a degradation product of the 50*S* subunit.

In the experiments we now report, cells of *E. coli* B(H) were grown in a tris-salt-glucose medium and labeled with H^3-lysine in the presence of chloramphenicol (0.7 μg/ml) during the exponential phase of growth. The crude extract of H^3-labeled cells was centrifuged on a density gradient of sucrose to separate the various precursor particles as well as the 50*S* and 30*S* subunits. The 40*S* region was centrifuged again in order to obtain a homogeneous 40*S* RNP free from other particles (Fig. 1a). It was dialyzed against $2 \times 10^{-4}M$ Mg^{++}, and freeze-dried. The preparation of the 40*S* RNP, whose protein moiety had been labeled with H^3-lysine, was dissolved in $5 \times 10^{-3}M$ acetate buffer (*p*H 5.6) containing $2.5 \times 10^{-3}M$ EDTA and 6*M* urea, and mixed with an appropriate amount of a preparation of the 50*S* subunit whose protein moiety had been labeled with C^{14}-lysine as a reference. The mixture (about 0.5 mg of ribosomes) was then incubated with pancreatic and T1 ribonucleases (1μg of each per milliliter) at 37°C for 15 minutes to remove rRNA from ribosomal proteins. After the digestion, about 3 mg of ribosomal protein was added. The sample was then adsorbed onto a carboxymethylcellulose column (0.5 by 30 cm) which had been equilibrated with a buffer composed of 6*M* urea and $5 \times 10^{-3}M$ acetate (*p*H 5.6) (*7*). The elution was done with an increasing (linear) concentration of acetate buffer from 0.05*M* to 0.5*M* at *p*H 5.6, containing 6*M* urea.

The elution pattern (Fig. 2a) demonstrated several clear differences between protein components of the 50*S* subunit and 40*S* RNP. The components *a*, *b*, and *d* present in the 50*S* subunit were lacking in the 40*S* RNP, and the relative amount of the peak *c* in the 40*S* RNP was only about two-thirds that of the 50*S* subunit. The peak *c* is often partially resolved into two peaks and has been separated into two (or three) components by disc electrophoresis, while *a*, *b*, and *d* showed a single component. This suggested that a component in the peak *c* was deficient in the 40*S* RNP. Thus the 40*S* RNP lacks at least four protein components as compared with the 50*S* subunit (*8*).

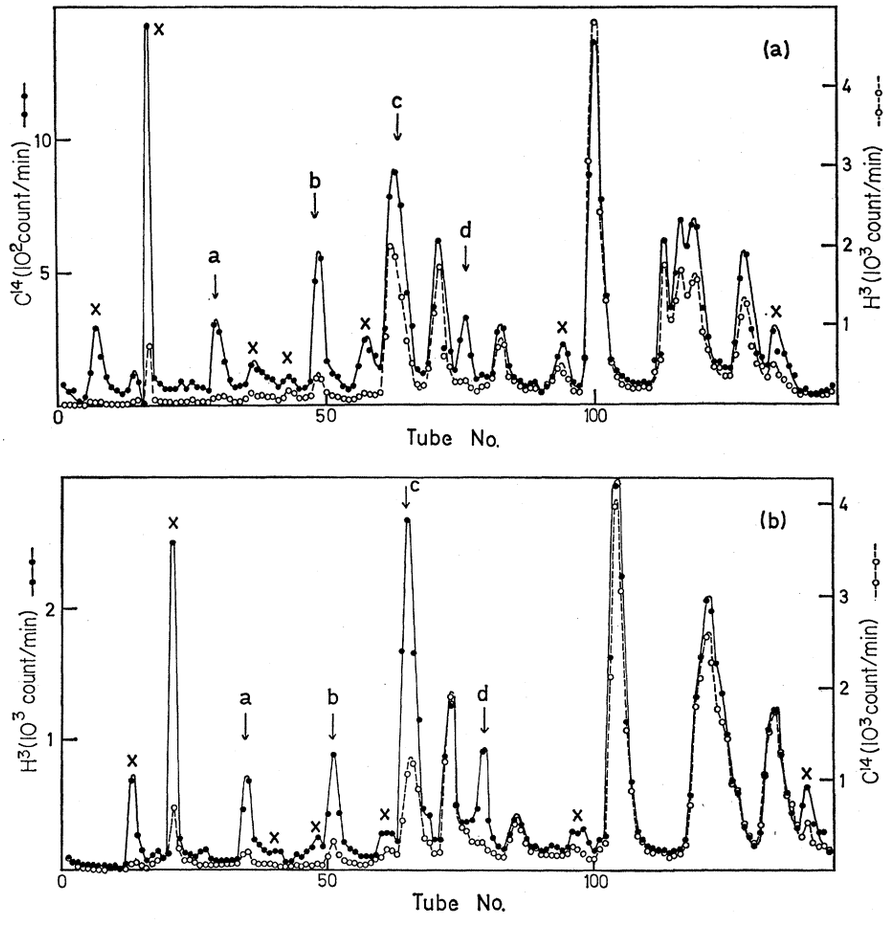

Fig. 2. Chromatography of ribosomal proteins on a carboxymethylcellulose column. (a) The 40*S* precursor RNP labeled with H^3-lysine (reference : the 50*S* ribosomal subunit labeled with C^{14}-lysine). (b) The physically produced 40*S* RNP labeled with C^{14}-lysine (reference : the 50*S* ribosomal subunit labeled with H^3-lysine).

The RNP having a similar sedimentation coefficient with the above 40*S* precursor RNP may be obtained by exposing the 50*S* subunit to a high concentration of CsCl solution as a result of removal of several ribosomal structural proteins (*9*). We have obtained a similar particle by treating the 50*S* subunit with 1.25*M* LiCl solution containing 0.1*M* Mg^{++} and $2.5 \times 10^{-2}M$ tris-HCl buffer at *p*H 7.4 (Fig. 1b). Its protein components were also analyzed on a carboxymethylcellulose column for the comparison with those of the precursor 40*S* RNP. In this experiment, C^{14}-labeled proteins of the 40*S* RNP were chromatographed with the H^3-labeled protein of the 50*S* subunit as a reference. The peaks *a*, *b*, and *d* were missing in this 40*S* RNP (Fig. 2b). The amount of the component *c* is also greatly reduced (*10*).

These results indicated that the 40*S* precursor RNP requires at least four protein components of *a*, *b*, *d*, and a component included in the peak *c* for its conversion into the 50*S* subunit. These components seem to be the same proteins which are released from the 50*S* subunit by salt treatment.

EIKO OTAKA
TAKUZI ITOH
SYOZO OSAWA

Department of Biochemistry and Biophysics, Research Institute for Nuclear Medicine and Biology, Hiroshima University, Hiroshima, Japan

References and Notes

1. B. J. McCarthy, R. J. Britten, R. B. Roberts, *Biophys. J.* **2**, 57 (1962); R. J. Britten, B. J. McCarthy, R. B. Roberts, *ibid.*, p. 83.
2. M. Iwabuchi, M. Kono, T. Oumi, S. Osawa, *Biochim. Biophys. Acta* **108**, 211 (1965).

3. R. E. McDonald, G. Turnock, J. Forchhammer, *Proc. Nat. Acad. Sci. U.S.* **57**, 141 (1967).
4. S. Osawa, *Progr. Nucleic Acid Res. Mol. Biol.* **4**, 161 (1965).
5. S. Osawa, in preparation.
6. A DEAE (diethylaminoethyl) treatment of the cells was done before the actinomycin D treatment according to the procedure of Leive [*J. Mol. Biol.* **13**, 862 (1965)].
7. The chromatography used here is a modification of Waller's method [*J. Mol. Biol.* **10**, 319 (1964)]. This method gives a fairly high resolution of each ribosomal protein component with a good reproducibility and recovery (E. Otaka, T. Itoh, S. Osawa, in preparation).
8. Although the peaks marked 'x' in the figure seem to show some quantitative differences between the 50*S* and 40*S*, these may be either degraded or coagulated ribosomal proteins, and they are not discussed in this paper.
9. M. Meselson, M. Nomura, S. Brenner, C. Davern, D. Schlessinger, *J. Mol. Biol.* **9**, 696 (1964); M. I. Lerman, A. S. Spirin, L. P. Cavrilova, V. F. Golov, *J. Mol. Biol.* **15**, 268 (1966); M. Nomura, P. Traub, *Proc. Symp. Rutgers*, in press.
10. In contrast to the precursor 40*S* RNP in which one component of peak *c* may be missing, this physically produced 40*S* RNP may be deficient in two protein components as judged from the ratio of H^3 to C^{14}. A milder treatment of the 50*S* subunit with LiCl might be necessary to obtain the particle having the same protein components with the precursor 40*S* RNP.
11. Supported in part by PHS grant GM 12045 and a grant from Matsunaga Science Foundation.

23 March 1967

Otolithic Membranes of the Saccule and Utricle in Man

Abstract. *The otolithic membranes of the human saccule and utricle can be prepared as whole mounts or surface specimens for microscopic examination. They are not simple, homogeneous, gelatinous structures as heretofore described. Instead, each shows a definite and characteristic fibrillar design, which appears to be correlated with the known cytoarchitectural pattern of the underlying neuroepithelium.*

The problems posed by weightlessness and other unusual stimuli to the vestibular system that are met with in space flight have led to something of a revival of learning about the structure and activity of the vestibular end organs. The saccular and utricular maculae, in their updated role of "gravireceptors" (*1*) or "linear translation sensors" (*2*), have been receiving attention such as they have not enjoyed since the work of Magnus and his school on the physiology of posture (*3*). During the past few years the neuroepithelia of these structures have been intensively scrutinized under the electron microscope (*4*), and their responses, along with those of the ampullar cristae of the semicircular canals, have been analyzed in control-system studies aimed at the development of mathematical models to represent their function (see *2*).

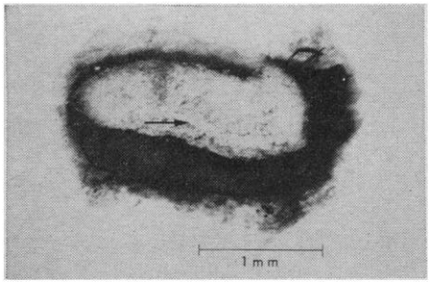

Fig. 1. Macula sacculi from an adult man (right ear). The white crystalline mass of otokonia still covers the otolithic membrane. The arrow indicates the "snowdrift" line, near the inferior margin, representing the heaviest deposit of otokonia. The surrounding membranes are stained with the OsO_4 fixative.

Although the otolithic membrane, the essential movable part of each macular end organ that overlies the neuroepithelium, was first pictured by Breschet (*5*) in 1836, it has remained, as in the time of Retzius (*6*), an ill-defined and vaguely apprehended "gelatinous layer," or, as Polyak has described it, "a jelly-like substance, of an almost homogeneous structure, in which the hairs of the haircells are embedded" (*7*). The reason for this vagueness lies in the unsatisfactory state of preservation of the otolithic membranes after routine histological treatment of the temporal bone for light microscopy, which, as reemphasized by Igarashi (*8*), involves shrinkage and distortion by powerful fixatives and acid decalcifying agents, and in the limited view of the membranes that is afforded by the usual stained cross sections. So far as we are able to determine from a painstaking search of the literature, the otolithic membranes, though always treated as familiar structures, have never been directly examined as separate entities comparable with the cupulae of the semicircular canals or the tectorial membrane of Corti's organ.

In a study of the normal and pathological anatomy of the human inner ear by direct microdissection in place of conventional microtomy, we have been able to prepare the otolithic membranes of the saccule and utricle for examination as whole-mounts or surface preparations. Their configuration is quite different from what has been universally assumed, and one may infer that it has important functional implications that are not taken into account by current vestibular theory.

Human temporal bones are obtained at routine autopsy by the standard method with the Stryker bone-plug cutter (*9*). The inner ear structures are fixed by perilymphatic perfusion through the oval and round windows with a buffered solution of 4 percent paraformaldehyde, stained in place for 1 to 2 hours with 0.5 to 1.0 percent solution of phosphate-buffered OsO_4 (Millonig), washed in physiological saline, and put through a graded series of alcohols up to 70 percent. Under the operating microscope the surrounding bone and the otic capsule are ground down to a thin shell with dental burrs and then removed. A final dissection of the membranous labyrinth is made with watchmaker's tweezers, iris scissors, and fine, electrolytically sharpened, tungsten-wire dissecting needles, as recently described (*10*).

This procedure affords an extraordinarily instructive view of the end organs, membranes, blood vessels, and nerve fibers of the inner ear.

The saccule and utricle are easily dissected out from the vestibule. The membranous portions surrounding the maculae are then removed. When the otokonia are still in place, the maculae (Figs. 1 and 2) look much the same as they have been shown for other species in Werner's (*11*) drawings and de Burlet and Hoffman's (*12*) stereophotographs. The characteristic raised

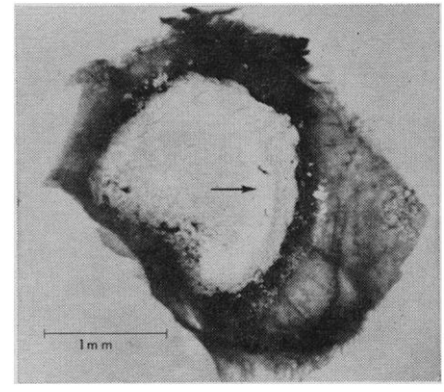

Fig. 2. Macula utriculi from an adult man (right ear) showing the horseshoe-shaped "snowdrift" line (arrow) along the lateral and anterior margins.