fat have been observed in developing inguinal fat.

Epididymal and ovarian deposits of white fat show patterns of development identical to that described for the inguinal tissue, but development commences at about 6 days of age. Multilocular cells have not been observed in these tissues.

To our knowledge the early stages of adipose tissue development in the hamster have not been studied previously, although a study of later developmental processes has been presented (*6*). The developmental patterns we have described do not correlate well with previous investigations in other species. There is no doubt that brown-fat cells are laid down in a tissue consisting primarily of unilocular cells. These unilocular cells develop without passing through a multilocular stage, as observed with the light microscope. They closely resemble similar cells found in white-fat deposits. If the unilocular cell types of scapular and inguinal deposits are indeed identical, and if the cells which give rise to brown fat represent a different cell type, as it appears, then we must conclude that brown fat is not related to a precursor form of white fat, but is laid down in a preexisting matrix of white fat, at least in the hamster. If this is a general phenomenon, it might indicate that brown fat is a younger tissue in evolutionary terms than white fat is. Recent studies tend to support this view. Some mammalian forms do not contain brown fat, but the presence of white fat appears universal in mammals. Nor do marsupials contain brown fat (*7*). Our studies of the developing opossum confirm this and demonstrate well-developed deposits of white fat in the scapular region normally occupied by brown fat in many mammalian forms. A large amount of additional work is needed both phylogenetically and ontogenetically before the view that brown fat arose after white fat can be finally accepted. In particular, extensive studies of the relations between the precursors of unilocular cells in the scapular and inguinal deposits are needed in the hamster. The relationships of these precursors to the IBF cell must also be established. The source of the IBF cell is uncertain, but the closeness of its association with the blood vessels may indicate that it arises from the endothelium of blood vessels. This has been previously demonstrated in the cold-adapting rat (*8*).

The source of lipid accumulated in the IBF cells is not known. It probably does not come from the unilocular cells, since these virtually disappear several days before the major lipid accumulation in the IBF cells at about 17 days. We have demonstrated a major accumulation of glycogen in brown fat at 16 days (*9*). This has been demonstrated in other tissues prior to lipid synthesis (*10*). The IBF cells do not store lipid at a time of apparently active storage in adjacent unilocular cells. This can be taken as strong circumstantial evidence that the unilocular cells appearing in the scapular deposits differ, at least physiologically, from the IBF cells deposited later. In addition it may indicate that the brown and white fat cells are not as closely related metabolically as has been previously assumed.

It is our opinion that a great number of uncertainties currently associated with development of adipose tissue can be overcome by studies of the hamster. In this species development of unilocular and multilocular cells is separated in time, and metabolic studies can readily be correlated with morphological events.

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Antibiotics in the Laboratory-Rearing of Cecropia Silkworms

Abstract. *A mixture of aureomycin and kanamycin prevents a fatal intestinal infection that usually occurs in all cecropia silkworms reared in the laboratory. Thus, for the first time, laboratory experimentation with these larvae and with those of other wild silkworms is practical.*

Studies of the growth and metamorphosis of the wild silkworm, *Hyalophora cecropia,* have been handicapped by the necessity of rearing the larval silkworms outdoors. When reared under laboratory conditions, the larvae invariably succumb to an intestinal disease characterized by diarrhea and the cessation of feeding; the disease routinely strikes in the fourth or early fifth instar. It then spreads rapidly throughout the entire stock despite one's maximal efforts to contain it. Staal (*1*) successfully reared cecropia by raising the temperature to 32°C. At Harvard, neither this nor any of numerous other regimens has met with any success.

Stress has been considered a promoter of intestinal infections in many insect larvae (*2*). The causative bacteria are normally present in small numbers in the gut. When the larvae are subjected to stress, changes in the microenvironment of the gut favor the growth and multiplication of these potential pathogens, resulting in an outbreak of a highly infectious disease. This theory, however, does not satisfactorily account for the rapid spread of the disease once it appears.

One thing is certain—stress is unavoidable in laboratory-rearing. Antibiotics have been used to prevent the outbreak of disease among some lepidopterans. Daily applications of streptomycin (*1*) and of streptomycin, aureomycin, terramycin, or tetracycline (*3*) have prevented disease in *Papilio polyxenes* and *Bombyx mori,* respectively. But, in our experience, neither streptomycin nor a mixture of penicillin, streptomycin, and aureomycin has been effective in protecting cecropia (*4*).

My attention was called to a previously untested antibiotic, kanamycin,

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.

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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*

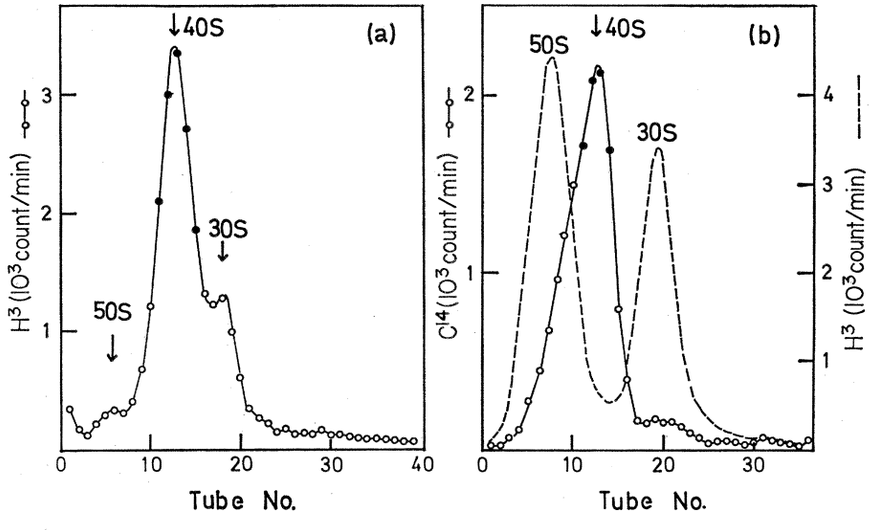

Fig. 1. Sucrose density-gradient centrifugations of the 40*S* precursor RNP and physically produced 40*S* RNP. (a) Sedimentation profile of H^3-lysine-labeled 40*S* region taken after the first sucrose density-gradient centrifugation. *Escherichia coli* cells were treated for 20 minutes with 0.7 μg of 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.