In the latter instance, it is apparent that marine bacteria are capable of attacking the less readily hydrolyzed polysaccharides and proteins of which detritus is undoubtedly composed (*7*).

The total quantity of detritus beneath a square meter of sea surface appears to be at least 500 g of dry organic material. Judging from the chlorophyll content of the water, only about 1 g of this can be attributed to living plant material. There is no obvious decrease in the amount of detritus or its protein content with depth after the first few hundred meters. Visual inspection gives little indication of the nature of the material although it is probably predominantly animal in origin.

The role of detritus in the marine food chain in this part of the ocean and in other open ocean areas of the world requires further investigation.

T. R. PARSONS
J. D. H. STRICKLAND
Pacific Oceanographic Group, Fisheries Research Board of Canada, Nanaimo, British Columbia

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22 January 1962

Insect Neurosecretory Material Separated by Differential Centrifugation

Abstract. The factor which accelerates the beating of the insect heart was found to be concentrated in a "large granule" fraction of the corpora cardiaca of the cockroach, *Periplaneta americana* (L.).

In a number of insect species, aqueous extracts of the corpora cardiaca have been shown to contain one or more factors which can markedly accelerate the heartbeat (*1*). Recent evidence indicates that the active factor may be a protein or a peptide (*2*). The object of the present investigation was to study the intracellular localization of this factor in the corpora cardiaca (*3*).

Corpora cardiaca, excised in the cold from adults of *Periplaneta americana* and dissected free of the corpora allata and other attached tissue, were rinsed and then accumulated in cold 0.4*M* sucrose. The corpora cardiaca from groups of 15 donors were homogenized in 0.2 ml of the sucrose solution, and then differentially centrifuged in 0.75-ml tubes, according to the schedule used by Blaschko *et al.* (*4*) for the mammalian adrenal medulla.

The milky homogenate was first spun for 10 minutes at 600*g*. The resulting supernatant was removed and recentrifuged for 20 minutes at 11,000*g*. The second centrifugation consistently yielded a compact bluish-white pellet (corresponding to Blaschko's "large granule" fraction). All of the centrifugations were carried out at about 2°C. For purposes of assay, each pellet was dispersed in an amount of sucrose solution identical in quantity and composition to that making up its supernatant. Each resuspended pellet and each supernatant was added to 3 ml of Yeager's saline (*5*), and frozen at −20°C until assayed.

Adult females of *P. americana* were used for the biological assay (*6*). The dorsal body wall, along with the attached heart, was placed in Yeager's saline in a tubular chamber (capacity 6 ml) adapted for perfusion and aeration. When the heart was beating at a steady rate, 1 to 2 ml of the test solution was introduced into the chamber with a hypodermic syringe. The heartbeat was then counted during alternate half-minute intervals until its increased rate had leveled off or started to decrease.

After the initial 600*g* centrifugation, heart-accelerating factor was found both in the supernatant and, in varying amounts, in the sediment. Centrifugation of this supernatant at 11,000*g* resulted in a sediment whose activity was markedly greater than that of either its own supernatant or the 600*g* sediment. Protein-nitrogen determinations (*7*) indicated that 10 to 70 percent more protein was present in the 11,000*g* supernatant than in the sediment, implying that activity in the sediment was not only greater but more concentrated.

The stability of the particulate localization of the factor was tested over short periods at various hydrogen ion concentrations. Sucrose (0.4*M*) was adjusted to each *p*H by the incorporation of various $10^{-2}M$ buffers, or by the addition of potassium hydroxide or hydrochloric acid. The pellet derived from the 11,000*g* centrifugation was resuspended in one of these solutions and recentrifuged at 11,000*g* for 20 minutes. The phases were then separated and tested as described above. The hypothetical particles appeared to be stable in sucrose within the *p*H range 6.2 to 9, but to lose the factor more or less completely to the supernatant at *p*H 3, 5, and 11. This sensitivity to acid conditions parallels that demonstrated for particles containing catecholamine from the adrenal medulla (*8*), for lysosomes from the mammalian liver (*9*), and for cytoplasmic granules from the rabbit polymorphonuclear leukocytes (*10*).

Several other highly active intracellular materials have been shown to be bound to particles, and current evidence suggests that these particles may often be identical with the electron-dense, membrane-limited vesicles conspicuous in electron micrographs of many neurosecretory and other organs (*11*). Such neurosecretory vesicles are plentiful in electron micrographs of cockroach corpora cardiaca (*12*), and also in electron micrographs which were made of their large granule fraction, obtained as described above by a single centrifugation at 11,000*g*. The vesicles appeared however to make up no more than about 40 percent (by volume) of the pellet. The color of this pellet is significant, since the bluish-white appearance of the corpora cardiaca themselves has frequently been attributed to their neurosecretory function. Moreover, Hodgson and Geldiay (*13*) were able to demonstrate a correlation between the disappearance of histologically detectable neurosecretory material from cockroach corpora cardiaca and the disappearance from the same organs of a factor capable of depressing the spontaneous nervous activity of the central nerve cord of the cockroach. The present work supports previous indications that the heart-accelerating factor of the corpora cardiaca is associated with neurosecretory vesicles.

J. J. T. EVANS
Biological Laboratories, Harvard University, Cambridge, Massachusetts

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11 December 1961

Actinomycin D Inhibition of Deoxyribonucleic Acid-Dependent Synthesis of Ribonucleic Acid

Abstract. Minute amounts of actinomycin D inhibit the synthesis of ribonucleic acid by nuclear extracts of HeLa cells in a ribonucleic acid–synthesizing system that is dependent on deoxyribonucleic acid and requires the presence of all four ribonucleoside triphosphates. The inhibition can be reversed by adding deoxyribonucleic acid to the enzymatic reaction. These findings support the work of others on the mode of action of actinomycin D in vivo.

Actinomycin D, one of the most potent anticancer agents discovered so far, exerts a profound influence on cellular nucleic acid (*1*). Actinomycin C forms complexes with DNA, and to a much lesser extent with RNA, similar to those formed by the acridine dyes with nucleic acids (*2*). It is by such complex formation that the antibiotic is thought to act. Although actinomycin is an inhibitor of bacterial DNA polymerase (*3*), in the bacterial or mammalian cell DNA synthesis appears to be unaffected by the antibiotic (*4*, *5*). However, Reich *et al.* have found a specific irreversible suppression of RNA formation in L cells exposed to this compound (*5*). Furthermore, their studies on mammalian cells infected with either RNA or DNA virus indicate that actinomycin D interferes with that RNA synthesis which is dependent on either cellular or viral DNA. The experiments reported here on the effect of antinomycin D on the cell-free syntheisis of RNA support such a mechanism.

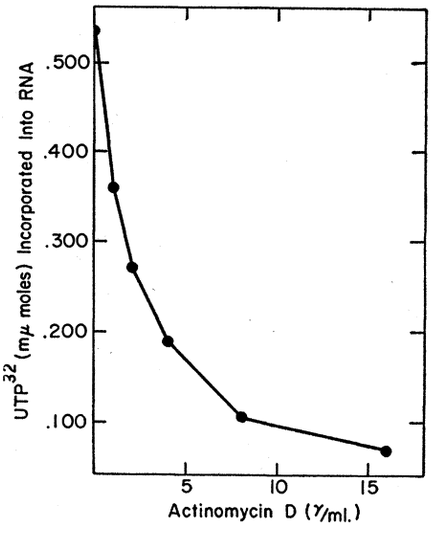

Fig. 1. Inhibition of incorporation of P^{32}-labeled uridine triphosphate (UTP^{32}) into RNA by varying concentrations of actinomycin D. Concentration of actinomycin D is given in micrograms (γ) per milliliter.

An RNA-synthesizing system that is dependent on DNA and requires the presence of all four ribonucleoside triphosphates has been found in nuclear extracts of HeLa cells (*6*). The enzymatically active component is composed of protein tightly bound with DNA and smaller amounts of RNA. This "aggregate" is capable of incorporating P^{32} from ribonucleoside triphosphate (labeled in its innermost phosphate) into RNA. In the experiments reported here proximally labeled uridine triphosphate was used to incorporate radioactive uridylic acid into RNA. The HeLa "aggregate" preparation, as well as the method of assay for RNA synthesis, have been described in a previous publication (*6*).

Small amounts of antinomycin D strikingly inhibited the incorporation of P^{32}-labeled uridine triphosphate into RNA (Fig. 1). The HeLa "aggregate" used in this experiment contained 1.44 mg of protein, 390 μg of DNA, and 144 μg of RNA. The reaction mixture contained 3 μmole of $MnCl_2$; 100 μmole of Tris buffer (*p*H 8.1); 20 μmole of NaF; 0.8 μmole each of adenosine triphosphate, guanosine triphosphate, and cytidine triphosphate; 0.1 μmole of uridine triphosphate-P^{32} (2.8×10^6 count/min μmole); and 0.1 ml of saturated $(NH_4)_2SO_4$ (4°C), at *p*H 8.0. The final volume was 1.0 ml, and incubation was for 20 minutes at 37°C. A similar curve of inhibition was obtained with P^{32}-labeled 5-ribosyluracil triphosphate (*7*). Actinomycin D was an effective inhibitor in this system at considerably lower concentrations than reported for the DNA polymerase (*3*). Mitomycin (100 μg/ml) and vancomycin (200 μg/ml) had no effect on the RNA-synthesizing system. With the same amount of "aggregate" with which 2 μg/ml of actinomycin D produced a 51-percent inhibition of incorporation of label into RNA, proflavine (200 μg/ml) caused a 35-percent and acriflavine (200 μg/ml) a 69-percent decrease in incorporation. In these experiments the RNA was isolated by repeated acid washes, since in the presence of high concentrations of acriflavine, ethanol precipitation of RNA was found to be incomplete.

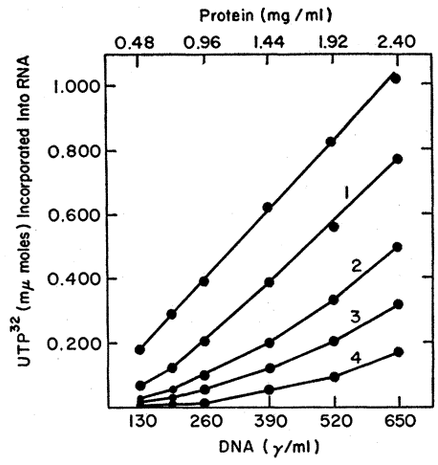

Fig. 2. Effect of varying the HeLa "aggregate" concentration on the degree of inhibition of RNA synthesis produced by different concentrations of actinomycin D. Actinomycin D concentrations: curve No. 1, 2 μg/ml; No. 2, 4 μg/ml; No. 3, 8 μg/ml; and No. 4, 16 μg/ml. Concentration of DNA is given in micrograms (γ) per milliliter.

The study of the kinetics of inhibition by actinomycin D is made difficult by the presence of considerable amounts of DNA bound to the HeLa RNA polymerase. It has been possible, however, to examine the effect of different concentrations of the antibiotic with

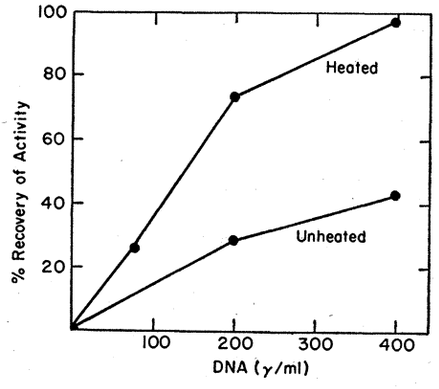

Fig. 3. Reversal of actinomycin D inhibition by addition of calf thymus DNA. Concentration of DNA is given in micrograms (γ) per milliliter.