ger reaction for activation is not an interaction between the cilia taking place while animals of complementary mating type are in loose contact. In the chemical induction of conjugation, paramecia of the same mating type conjugate with each other without showing a prior mating reaction, indicating that the agglutinative reaction is not an absolute prerequisite for the occurrence of conjugation (*12*). Therefore, the conclusion that cilia participate in the mating reaction and the demonstration that dead *P. caudatum* can induce living animals of the opposite mating type to conjugate do not necessarily mean that cilia can activate paramecia to conjugate.

The experiments reported here make this point clearer, for they demonstrate that the cell-free preparation predominantly composed of cilia can induce conjugation in paramecia of the opposite mating type; they thus indicate that isolated cilia not only adhere to paramecia of the opposite mating type but can also activate them to conjugate.

Since both agglutination and activation should have a material basis, substances participating in agglutination, and others participating in activation, may be postulated. The former have been called mating type substances and are believed to be proteins (*5*, *6*). Neither they nor activation substances have yet been extracted in soluble form.

These two kinds of substances may be identical as Metz (*5*) postulated. According to his postulation, the interaction between mating type substances themselves is the trigger reaction which activates paramecia to conjugate. The present experiments do not prove this but they do at least indicate that both phenomena are due to substances on the same organelles, the cilia.

The problem would be solved if these substances could be extracted and compared. Such studies may be facilitated by using the method reported here, since it yields cell-free preparations which possess strong activities of both kinds, specific adhesion to cells of complementary type and activation for conjugation.

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Mitomycin C: Effect on Ribosomes of Escherichia coli

Abstract. *Sucrose-gradient analyses of cell-free extracts of* Escherichia coli *B show that the sedimentation patterns are markedly affected by exposure of the cells to mitomycin C; 50S subunits are progressively degraded in the cells after 30 minutes exposure. The 30S subunits, although affected, are less sensitive to the antibiotic than the 50S subunits. Incorporation of uracil-2-C^{14} into the ribosomal particles by the treated cells is markedly reduced by exposure.*

Several reports (*1*, *2*) suggest that the antibiotic mitomycin C (MC) has a specific effect on cellular DNA. Other studies indicate that its effect is not entirely limited to DNA (*3*, *4*). Kersten and Kersten (*5*) have found that MC causes primarily the breakdown of RNA and suggest that breakdown of DNA may be a secondary reaction. Our studies on the mode of action of MC in *Escherichia coli* also show that the inhibitory action is more complex than first believed. We now report that ribosomes in cells of *E. coli* B are degraded during exposure to MC. Evidence is also presented indicating that the ability of ribosomes to incorporate uracil-2-C^{14} is impaired in exposed cells.

Exponentially growing [in a glucose-synthetic medium (*6*)], cells of *E. coli* B were washed with 0.05*M* phosphate buffer, *p*H 7.0, suspended in 100 ml of the same culture medium at a concentration of 5×10^8 cells per milliliter, and incubated at 37°C in the presence of MC, with aeration. After treatment the cells were harvested, washed twice with 0.01*M* tris-HCl buffer (*p*H 7.3), and suspended in 5 ml of the same

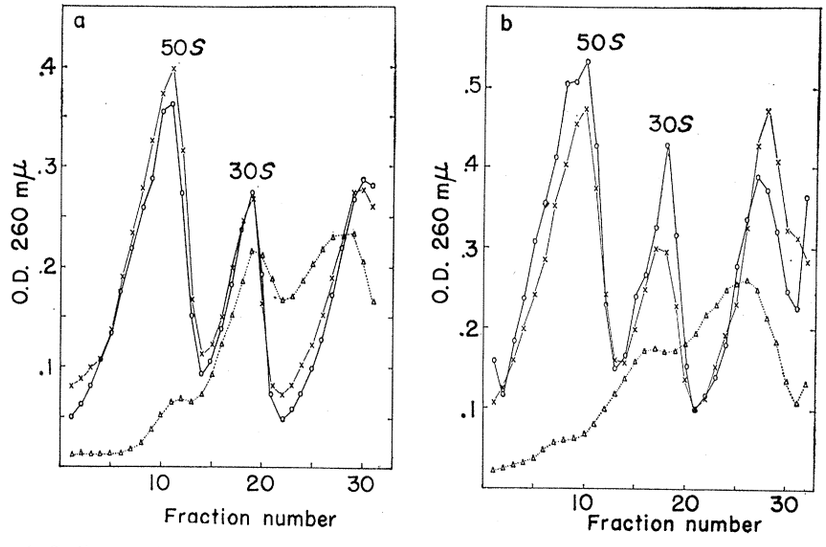

Fig. 1. Sedimentation profiles of extracts of *Escherichia coli* B obtained after 60 minutes (*a*) and 90 minutes (*b*) treatment with MC. Cells were incubated in the absence of MC as controls (o——o), in the presence of MC at 0.1 μg/ml (×——×), and in the presence of MC at 5.0 μg/ml (Δ . . . Δ). Crude extract (0.5 ml) in 0.01*M* tris-HCl buffer containing $10^{-4}M$ magnesium acetate, *p*H 7.3, was layered on the top of 4.5 ml of sucrose in a linear density gradient (5 to 20 percent sucrose in 0.01*M* tris-HCl buffer and $10^{-4}M$ magnesium acetate, *p*H 7.3) in cellulose centrifuge tubes. Each preparation was centrifuged at 100,000*g* for 120 minutes at 4°C in a swinging-bucket rotor (SW 39) in a Spinco model L ultracentrifuge. Each tube was then punctured at the bottom, and 10-drop fractions were collected and diluted to 3.0 ml for optical density measurements at 260 mμ.

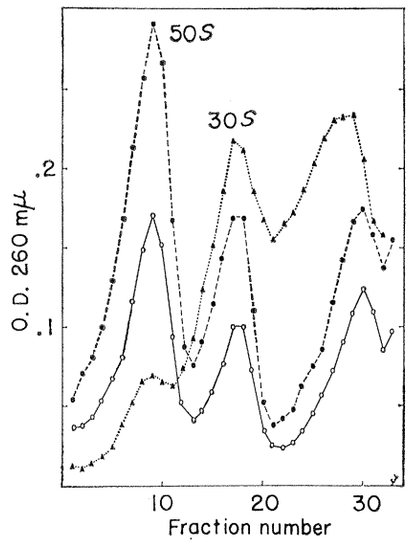

Fig. 2. Changes, with time, of sedimentation profiles of extracts treated with MC at 5 μg/ml. Extracts in $10^{-4}M$ magnesium acetate were prepared from cells taken: immediately after the addition of MC (o———o), after 30 minutes (●----●), and after 60 minutes (▲ . . . ▲). Sedimentation analyses as described for Fig. 1.

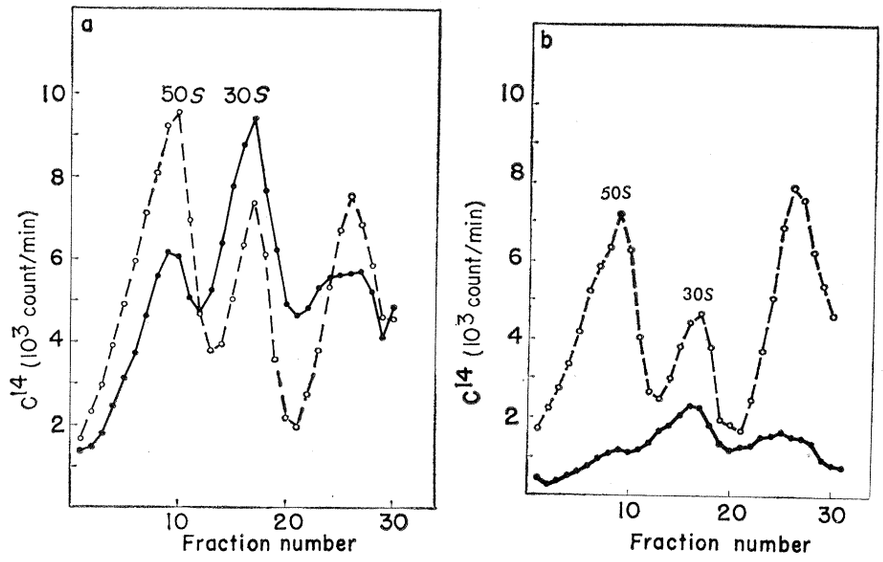

Fig. 3. Effect of MC on the incorporation of uracil-2-C^{14} into ribosomes: MC-treated, ●———●; control, o — — — o. Cells treated with MC (5 μg/ml) for 30 minutes (*a*) and 60 minutes (*b*) were labeled (pulse) with 2.5 μc of uracil-2-C^{14} per 100 ml of culture for 60 seconds, and then treated (chase) with uracil (50 μg/ml) for 15 minutes. Cells were then washed and extracts were prepared in 0.01*M* tris-HCl, *p*H 7.3, containing $10^{-4}M$ Mg acetate. Centrifugation as described for Fig. 1. Radioactivity measurements were made with a Packard tri-carb liquid scintillation counter.

buffer with $10^{-4}M$ magnesium acetate added. Crude extracts of the cells were prepared with a French pressure cell. After removal of the cell debris by centrifugation at 20,000*g* for 15 minutes, samples were subjected to sucrose density gradient analyses (*7*).

The MC had no apparent effect on the distribution of the 30*S* and 50*S* components during the first 30 minutes of treatment. Analysis in the presence of $10^{-2}M$ Mg^{++} showed the distribution patterns of the polysomes and the 70*S* ribosomes to be identical with those from the control culture; it appeared that the ribosomes were not affected by MC during the first 30 minutes of treatment. However, the lethal dose of MC (5 μg/ml) had pronounced effects on the distribution of ribosomes in cells treated for more than 30 minutes (Fig. 1). The pattern in cells after 60 minutes of treatment showed almost complete degradation of the 50*S* subunits, with concomitant accumulation of lighter fractions (Fig. 1*a*). The 30*S* subunits were also degraded, but they were less sensitive to MC and disappeared later than the 50*S* particles (Fig. 1*b*). Sedimentation analyses in the presence of $10^{-2}M$ Mg^{++} of the extracts from cells treated for 60 minutes showed the presence of 70*S* ribosomes, although the amount was much less than in the control; peaks corresponding to the polysomes were not detected. Thus, the 30*S* and 50*S* components remaining after 60 minutes of treatment with MC (5 μg/ml) were able to associate into 70*S* particles but were unable to form polysomes.

A lower concentration of MC (0.1 μg/ml or less), causing only filament formation without any apparent effect on macromolecular metabolism of the treated cells, had no effect on ribosomes.

Figure 2 shows that the 50*S* subunits were preferentially degraded after 60 minutes of treatment with MC (5 μg/ml), while the 30*S* components still showed some increase. Apparently the degradation of ribosomes is not due to the direct interaction of MC with the ribosomes: incubation of a crude extract with a very high concentration of MC (100 μg/ml) did not influence the ribosomal profile and no association of MC with any fractions of ribosomes was detected.

In "short-pulse" experiments with uracil-2-C^{14} (*8*), the amount of C^{14} incorporated into the lighter fractions by cells treated with MC (5 μg/ml) for 30 minutes was much less than incorporation by the control; almost no incorporation was observed in cells treated for 60 minutes. This indicates that the ability of treated cells to synthesize cellular RNA is somewhat impaired by MC even after a 30-minute exposure, although the ribosomal distribution pattern is not affected at that time. To confirm this, chase experiments (*9*) were carried out (Fig. 3). After a short pulse of uracil-2-C^{14} and a subsequent chase of 15 minutes, the label was mainly associated with the 30*S* subunits, being significantly less in the 50*S* fraction than in the 30*S*. The amount of label incorporated into ribosomes of cells treated for 60 minutes was very low, though the distribution was similar to that of cells treated for 30 minutes. On the other hand, in the control cells both the 30*S* and 50*S* particles were equally labeled, and the distribution pattern was identical with that of the absorbancy at 260 mμ. Thus, in addition to the degradation of ribosomes, MC at a dosage of 5 μg/ml also inhibits the synthesis of ribosomal RNA. The 50*S* components are particularly sensitive to the drug.

Similar results with the inhibition of synthesis of ribosomal RNA have been reported recently in cells of *E. coli* exposed to ultraviolet irradiation (*10, 11*).

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Methylurea and Acetamide: Active Reabsorption by Elasmobranch Renal Tubules

Abstract. *The renal tubules of the shark actively reabsorb urea. They also can reabsorb acetamide and methylurea, but there is no evidence for active reabsorption of thiourea. The specificity of the transport system thus appears to be different from the urea secretory system in the frog in which thiourea is secreted but acetamide and methylurea are not secreted.*

The renal tubules of elasmobranchs actively reabsorb urea from the tubular fluid with the result that the concentration of urea in the urine is lower than that of plasma (*1–3*). This mechanism helps in maintaining a high concentration of urea in the blood (approximately 350 m*M*/liter) which in turn makes it possible for the elasmobranch to be in osmotic equilibrium with the surrounding seawater in spite of lower concentrations of electrolytes in the plasma.

We have studied the renal excretion patterns of compounds related to urea in chemical structure. This was done in order to be able to compare the carrier specificity of the urea transport system in the shark tubules with that of other species, such as frog and mammal. The compounds used were methylurea ($CH_3NHCONH_2$), acetamide (CH_3CONH_2) and thiourea (NH_2CSNH_2).

Ten sharks, representing five species of elasmobranchs, were studied (*4*). A polyethylene catheter was inserted into the urinary papilla in the females or urogenital papilla in the males and tied securely in place. A narrow side tube opened into the part of the catheter nearest the papilla. By blowing air through the side tube the catheter could be emptied at the end of each urine collection period. The shark was placed in running seawater in a narrow wooden box. The C^{14}-labeled test compounds, together with the same nonlabeled compounds, were dissolved in seawater and injected with a hypodermic needle into the caudal vein or artery 2 to 3 hours prior to the first urine collection period. When inulin was given, it too was injected into the caudal vein 3 hours prior to the first urine collection. Blood samples from the caudal vein or artery were taken before and after each urine collection period, which lasted from 1 to 12 hours. (The length of the collection period had no effect on the results.) The urine and blood samples were analyzed for urea by the Conway method; for inulin, by the diphenylamine method; and the C^{14}-labeled test compounds, by liquid scintillation spectrometry.

Data for all fish studied are presented in Table 1. In four sharks (three different species) injected with methylurea, the ratio of the methylurea concentration in the urine (U_m) to that in the plasma (U_m/P_m) was less than unity in all 11 clearance periods (average, 0.62). In three sharks injected with acetamide, the ratio for acetamide, U_a/P_a, was likewise lower than unity in all nine periods (average 0.76). The ratio for urea, U_u/P_u, was also less than unity and less than the simultaneously measured ratios U_a/P_a and U_m/P_m in all collection periods.

Table 1. Concentration of urea or test compound in plasma (P). Concentration of compound in urine divided by its concentration in plasma (U/P). The species *Heterodontus francisci* and *Rhinotriacis henlei* were caught in the coastal waters of Baja California; the species *Mustelus canis* and *Squatina dumerili*, in the coastal waters of North Carolina; and *Squalus acanthias*, in the coastal waters of Maine.

Species	Period	Test compound		Urea	
		P (mM/l)	U/P	P (mM/l)	U/P
		Methylurea			
Squatina dumerili	1	10.5	0.67	376	0.44
	2	10.2	.64	374	.47
	3	9.9	.70	378	.48
	4	9.8	.73	376	.54
Mustelus canis	1	21.7	.82	288	.68
Mustelus canis	1	21.0	.72	273	.57
Squalus acanthias	1	10*	.49		
	2	10	.52		
	3	10	.56		
	4	10	.60		
Squalus acanthias	1	1.0*	.39		
Average			0.62	343	0.53
		Acetamide			
Heterodontus francisci	1			367	0.20
	2	0.01	0.84	368	.20
	3	.01	.76	370	.23
Heterodontus francisci	1	.01	.75	419	.14
	2	.01	.68	397	.18
	3	.01	.77	402	.16
Rhinotriacis henlei	1	.01	.76		
	2	5.8*	.89	345	.60
	3	5.8*	.68	341	.34
	4	13.5*	.71	341	.39
Average			0.76	372	0.27
		Thiourea			
Rhinotriacis henlei	1	0.01	2.69	323	0.56
	2	.01	2.85	315	.56
	3	.01	3.25	313	.57
Mustelus canis	1	20.0	2.15	296	.89
Average			2.74	312	0.64

* Represents approximate plasma concentration, calculated from the amount injected and the estimated total body water.