of these substrates in response to small changes in arterial glucose. If verified by more direct evidence, the possible relations of such a center(s) to appetite regulation and normal weight maintenance will be of great interest.

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Streptomycinoid Antibiotics: Synergism by Puromycin

Abstract. *Puromycin synergizes the lethal action of streptomycin and related antibiotics. This is interpreted to mean that puromycin action uncovers a sensitive site (or sites) on the 30S ribosome. The streptomycinoid antibiotics can then associate more readily with the ribosome and inhibit further synthesis of valid protein.*

The antibiotics streptomycin, kanamycin, neomycin, paromomycin, gentamycin, viomycin, and hygromycin B all strongly inhibit polypeptide synthesis in cell-free bacterial extracts (*1*), and all possess certain common features in their chemical structures (*2*). Mutants of microorganisms that are resistant to one of these "streptomycinoid" antibiotics frequently show resistance to others in the group (*3*). These observations and those of other investigators suggest a similarity in the mode of action of these antibiotics (*4*). In this report we provide evidence that all members of this group are bactericidal against *Escherichia coli* (following a brief lag) and that this action is antagonized by chloramphenicol and synergized by puromycin, although both of these antibiotics inhibit protein synthesis. Interpretation of this evidence indicates that the streptomycinoids are closely similar in their lethal mode of action.

The general features of the lethal action of the streptomycinoids are illustrated in Fig. 1. These data were obtained using gentamycin, but quite similar curves have been obtained with streptomycin and the other streptomycinoids (Table 1). With gentamycin alone (Fig. 1, curve *C*) a characteristic lag is followed by exponential killing of the microorganisms. There is also a final period during which the survivors die more slowly. In the presence of either chloramphenicol or puromycin alone viability is constant or rises slightly during the first 2 hours (not shown). The antagonism of the lethal effect by chloramphenicol (curve *A*) and the synergism by puromycin (curve *D*) present a striking contrast. After the first 20 minutes puromycin antagonizes further killing by gentamycin. This late antagonism by puromycin can be enhanced by incubating the bacteria with puromycin for 60 minutes before adding gentamycin (curve *B*). Chloramphenicol does not give a synergistic effect at any concentration.

Although the best evidence for the mode of action of a streptomycinoid antibiotic has been obtained for streptomycin itself, we propose that the model presented below for streptomycin action is valid for the entire group. Streptomycin inhibits bacterial protein synthesis in growing cultures (*5*) as well as in extracts, but, since many other biological effects of streptomycin have been described, there has been some question whether the inhibition of protein synthesis per se is, in fact, its lethal action (*4*). The existence of the lag and of the antagonism by chloramphenicol have even been interpreted to mean that protein synthesis is actually required for expression of the lethal action of streptomycin. The puromycin synergism contradicts this idea and clearly calls for an interpretation based on events at the ribosome, which is the common site of action of all three antibiotics. The ribosome may be thought of as operating in a cycle (Fig. 2): the free ribosome associates with a molecule of messenger RNA; a polypeptide

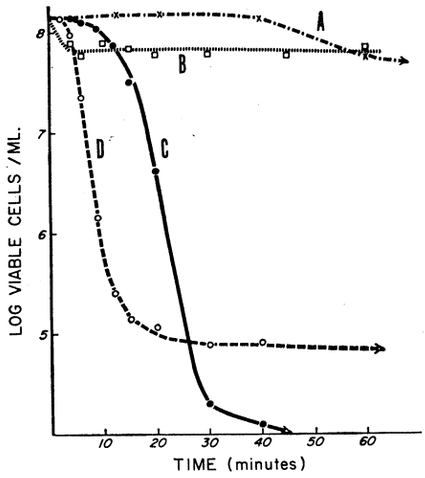

Fig. 1. A phenylalanine-requiring mutant of *Escherichia coli* strain 15 was grown exponentially at 37°C in a tris-buffered minimal medium (*17*) containing glucose and phenylalanine (generation time, 50 minutes). Antibiotics (*18*) were added as follows: *A*, gentamycin and chloramphenicol at 0 minutes; *B*, puromycin, 60 minutes before the addition of gentamycin, and gentamycin at 0 minutes; *C*, gentamycin at 0 minutes; *D*, gentamycin and puromycin at 0 minutes. Concentrations: gentamycin, 10 μg/ml; puromycin, 500 μg/ml; and chloramphenicol, 200 μg/ml. Serial dilutions of the samples were made in ice cold saline and were plated on Difco nutrient agar.

chain is initiated, grows, and is released; and messenger RNA is also ultimately released (*6*). Streptomycin (or other streptomycinoids) may be postulated to associate irreversibly with the ribosome, thereby eliminating that ribosome as a further site of protein synthesis. It is the irreversibility of this association that is lethal. In addition it must be postulated that this irreversible association occurs only when the ribosome is in a particular, sensitive state, which we take to be the free state (*7*). These postulates lead to the following interpretation of streptomycin action.

When streptomycin is added to an exponentially growing culture, only a few of its 10^4 ribosomes are in the sensitive state. These ribosomes are quickly inactivated, but the cell remains viable until a sufficient portion of its ribosomes have cycled around to the sensitive state and have been inactivated in turn. The inactivated ribosomes can still associate with messenger RNA (*7, 8*), but evidently cannot catalyze the normal synthesis of protein; as a result the cell can no longer form a colony on nutrient agar and is by definition nonviable. Chloramphenicol apparently holds a large fraction of the ribosomes in insensitive states by a mechanism

which is not yet understood (*9*), thereby preserving them from inactivation by streptomycin. Hence both the existence of the lag and the chloramphenicol antagonism are accounted for.

Puromycin inhibits protein synthesis by stripping the unfinished peptide chain prematurely from the ribosome (*10*). Recent evidence suggests that when this happens the ribosome also leaves the messenger RNA (*11*). The ribosomes revert to the free state (via the "puromycin shunt" in Fig. 2) and may be rapidly inactivated by streptomycin when this antibiotic is also present in the cell. Thus the observed synergism by puromycin is predicted by the model. The streptomycin-sensitive site (or sites?) is known to be on the 30*S* ribosome (*7*, *8*), and this is also the ribosome with which messenger RNA probably binds (*12*), while the growing peptide is on the 50*S* ribosome (*6*). Therefore, it is probably the release of messenger RNA, rather than the release of the peptide, which reveals the sensitive site. Since chloramphenicol inhibits the action of puromycin both in vitro (*13*) and in vivo (*11*), when both antibiotics are used simultaneously with streptomycin one should expect to find no killing, as shown by the block of the puromycin shunt (Fig. 2); and this was observed (Table 1).

The apparent requirement for protein synthesis is interpreted as a requirement that the sensitive site be cleared before streptomycin can associate with it. The foregoing considerations strongly suggest that for all streptomycinoids the sensitive site is concealed during normal protein synthesis, probably by messenger RNA. It is not yet clear whether the same site is involved in each case.

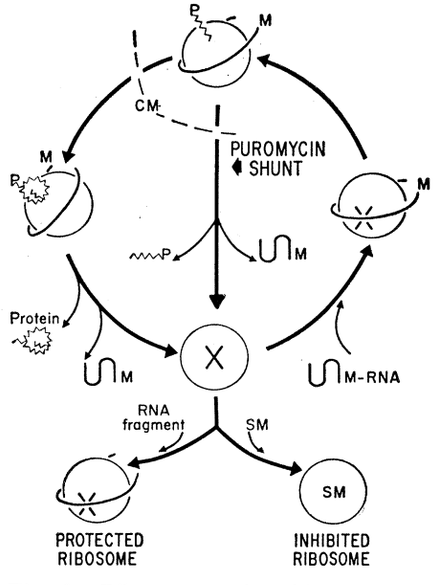

Fig. 2. Ribosome cycle with postulated points of interaction with antibiotics. *X*, the streptomycin-sensitive site; *CM*, chloramphenicol; *SM*, streptomycinoid; *M*, messenger RNA; and *P*, polypeptide chain.

Table 1. Puromycin synergism and chloramphenicol antagonism of streptomycinoid antibiotics. The indicated antibiotics were added at zero minutes to a culture of a phenylalanine-requiring mutant of *Escherichia coli* strain 15, growing exponentially as described in the legend of Fig. 1. Samples were removed at the times indicated and were plated on Difco nutrient agar. CM, chloramphenicol (200 μg/ml); Puro, puromycin (500 μg/ml); SM, streptomycin (40 μg/ml); PM, paromycin (50 μg/ml); NM, neomycin C (20 μg/ml); KM, kanamycin (100 μg/ml); VM, viomycin (1000 μg/ml); and HM, hygromycin B (1000 (μg/ml).

Antibiotic	Additions		Time (min)	Viable cells/ml	Survivors (%)
	CM	Puro			
None	−	−	0	1.0×10^8	100
	−	−	50	2.1×10^8	210
SM	−	−	10	1.1×10^8	110
	−	−	60	4.0×10^5	0.4
	−	+	10	5.0×10^6	5
	+	−	60	1.2×10^8	120
	+	+	60	1.0×10^8	100
PM	−	−	10	9.0×10^7	90
	−	−	60	1.5×10^4	0.015
	−	+	10	1.0×10^6	1
	+	−	60	1.1×10^8	110
NM	−	−	4	9.0×10^7	90
	−	+	4	3.0×10^6	3
	+	−	60	1.1×10^8	110
	+	+	60	1.1×10^8	110
KM	−	−	10	7.0×10^6	7
	−	−	60	4.0×10^4	0.04
	−	+	10	1.5×10^5	0.15
	+	−	60	1.1×10^8	110
VM	−	−	30	5.0×10^7	50
	−	+	30	2.5×10^5	0.25
	+	−	30	1.0×10^8	100
HM	−	−	10	6.0×10^7	60
	−	−	60	7.0×10^3	0.007
	−	+	10	4.5×10^4	0.05
	+	−	60	3.5×10^7	35

The late antagonism by puromycin can perhaps be accounted for by supposing that the RNA which accumulates during puromycin inhibition (*14*) is in part degraded messenger RNA that can envelop the ribosomes in a quasi-normal fashion, once again protecting the ribosome. The final deceleration in rate of killing by streptomycin (in the absence of puromycin) could be accounted for by a similar mechanism, since there is some accumulation of messenger-like RNA even in streptomycin inhibition (*15*).

Although there are differences in the responses of various strains of *Escherichia coli* to inhibition by the streptomycinoids, the same features are generally observable. For example, in inhibition by puromycin plus paromomycin, strain B shows the synergism to a much lesser degree and the late antagonism to a greater degree than strain 15, while strain W is intermediate in its response.

A final point, which is of considerable potential utility, is that according to our model the rate of killing by streptomycinoids is an indicator of the rate at which ribosomes are arriving at the sensitive state; and thus the shape of viability curves, under various conditions of streptomycinoid inhibition, is a reflection of events occurring at the ribosome (*16*).

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Transferrin: Variations in Blood Serum of Red Howler Monkeys

Abstract. *Blood serum samples from 33 red howler monkeys* (Alouatta seniculus) *were examined. Three different phenotypes were found and denominated A, B, and C. Four serums could not be classified because their transferrin apparently did not bind iron-59, possibly owing to saturation. A difference was observed in the electrophoretic migration and pattern of the transferrins in these monkeys compared with those of other primates.*

The fact that transferrin of human blood is characterized by a genetic polymorphism (*1*) has led to the knowledge that *Macaca mulatta* (*2–4*), *M. irus* (*2, 3*), *M. radiata* (*2*), *M. nemestrina* (*2*), *Papio doguera* (*5*), and *Cebus nigrivitattus* (*3*) have a more extensive polymorphism than man. Multiple transferrins were also found in the chimpanzee (*2; 6–8*) and their genetical mechanisms have been already established (*9*). Similar information about other pongids (*2*) has been obtained, but the transferrins of other primates have not been studied, particularly those of the platyrrhines. Information about New World primates is in general very scanty, and the new trends in research, as described by Buettner-Janusch (*10*), have barely been aimed at these animals.

This report refers to a study made in 33 howler monkeys (*Alouatta seniculus*) collected in central Venezuela. Howler monkeys have peculiar vocal specializations. They belong to the infraorder Platyrrhina (*11*) and are found all over tropical America. Thirty-one of these animals were kept in the animal house of our institute, and two belonged to the local zoo (*12*). Blood was drawn by puncture of either cardiac or femoral vein under sterile conditions. For the handling of these samples we used routines which have given optimum results in our laboratory. All samples were kept at −20°C before electrophoresis. Some serums were stored for as long as 16 months, most for 4 or 5 months, and others for only 1 day before analysis.

Transferrins were studied by starch-gel electrophoresis in borate buffer by Smithies' horizontal technique (*13*) with minor modifications (*14*). Identification was made with iron-59 in sulfate form added to the serum in a proportion of 5 μc/ml, and autoradiography was performed on Ansco non-screen x-ray films (*15*). Protein fractions were stained with amido black 10B.

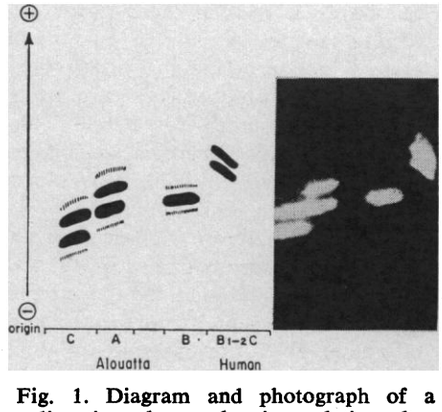

Fig. 1. Diagram and photograph of a radioactive plaque showing relative electrophoretic mobilities of transferrin bands in howler monkeys and man.

Four of the animals could not be classified because their transferrins did not bind iron-59, possibly because of saturation. Unfortunately, we did not have enough serum from these specimens to repeat the study. In those red howler monkeys where the transferrin could be made visible by autoradiography, results indicated three well-defined phenotypes, one constituted by three bands and the other two by four bands differing only in their mobility. Since there was no information about breeding and pedigree, the phenotypes were designated by letters (Fig. 1). Eight specimens were type A (27.6 percent), 20 were type B (68.9 percent), and only one was type C (4.3 percent).

The pattern of slow-moving multiple bands presented by the howler transferrins may suggest that our results are due to the presence of neuroaminidase in these serums. The splitting and slowing of transferrin bands as a consequence of contamination with agents that produce neuroaminidase has been mentioned (*16*). This possible effect was controlled with the blood of the two animals from the zoo. Their bloods, obtained and handled under checked sterile conditions, were the last studied. The serums were analyzed 30 hours after exsanguination of the animals; one of the serums was of type A and one of type B. We conclude that none of our three phenotypes are due to the action of neuroaminidase.

The phenotype patterns in this species of *Alouatta* are somewhat similar to those obtained by Boyer and Young (*6*) and Buettner-Janusch (*7*) in chimpanzees. The howler monkey transferrins move in the same general zone as those of the chimpanzee—that is, they are slower than human transferrin C.

From the meager comparative data available for transferrins in platyrrhines it might be concluded that the three howler monkey phenotypes are different from those of *Cebus* (*2, 3*), *Lagothrix* and *Ateles* (*2, 17*), and *Cacajao* (*2*). They also differ from those of all known prosimians and cercopithecoids. These serological data