homocystinuric liver did not inhibit the cystathionine synthetase activity of control liver (Table 1). Radioactivity from authentic L-cystathionine-C^{14} incubated with the extract of homocystinuric liver under the standard assay conditions was recovered entirely with cystathionine. It was demonstrated by means of paper chromatography that the substrates for the assay of cystathionine synthetase, serine-3-C^{14} and homocysteine were still present after incubation with the extract of homocystinuric liver. The specificity of the deficiency of cystathionine synthetase activity is suggested by the adequate activity of methionine activating enzyme. Since the reaction mixture for assay of cystathionine synthetase contained added pyridoxal phosphate, the failure to detect cystathionine synthetase activity cannot be attributed to a deficiency of this cofactor.

A major metabolic pathway for methionine is thought to be conversion to cyst(e)ine and its end products (*6*). The homocystine (and methionine) excreted in the urine of homocystinuric patients accounts for only a small portion of the total intake of methionine. If the block in cystathionine synthetase is complete in these patients, they must metabolize the major portion of the ingested methionine by alternate pathways.

Because cystathionine formation is a necessary step in the major mammalian pathway for cysteine biosynthesis, there is a strong likelihood that a homocystinuric child will suffer from cysteine deficiency during the neonatal period when dietary intake of protein is much lower relative to the body's anabolic needs than it is in later years. It is conceivable that a homocystinuric infant fed human milk may receive adequate cystine whereas such a child fed cow's milk, known to be much poorer in this amino acid (*2*), would not receive adequate amounts. In either case, early dietary cyst(e)ine supplementation may prevent irreparable damage. Another possibility which should be considered is that cystathionine, reported to be present in high concentration in human brain (*7*), serves some necessary function in addition to that of precursor of cysteine. If this is so, dietary cystathionine supplementation might be helpful. Whether abnormal accumulation of homocyst(e)ine, methionine, or one of their metabolites contributes to the pathological manifestations of homocystinuria and would thus warrant methionine restriction also remains to be determined.

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Erythromycin: Mode of Action

Abstract. *Erythromycin, a specific inhibitor of protein biosynthesis, inhibited the incorporation of phenylalanine by a cell-free ribosomal system prepared from* Escherichia coli.

The antibiotic, erythromycin, inhibits the biosynthesis of protein in susceptible bacteria but permits continued synthesis of nucleic acids (*1*); this mode of action has been compared by Brock and Brock (*2*) to that of chloramphenicol. Antibiotics which are specific inhibitors of protein synthesis in vivo have been shown to inhibit the incorporation of radioactive amino acids into trichloracetic acid–insoluble polymers by cell-free ribosomal systems prepared from antibiotic-sensitive bacteria; this has been demonstrated for chloramphenicol (*3*), puromycin (*4*), tetracyclines (*5*), and streptomycin (*6*). Inhibition of amino acid incorporation in a cell-free system by chloramphenicol is generally regarded as circumstantial evidence that such a system represents a valid model of protein biosynthesis.

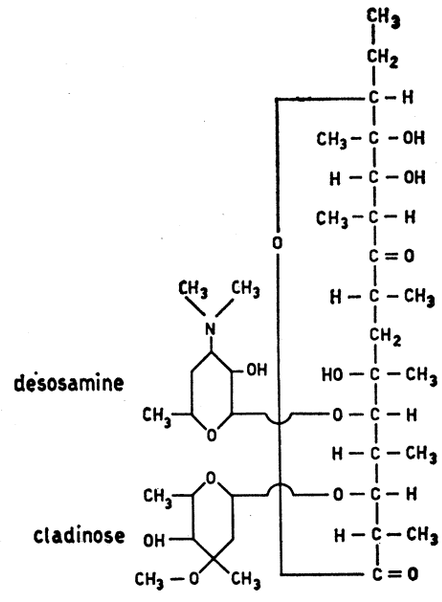

Fig. 1. Erythromycin.

We wish to report that erythromycin also inhibits the formation of polyphenylalanine in a cell-free ribosomal system prepared from *Escherichia coli* after the method of Matthaei and Nirenberg (*7*). Experimental details and results are given in Table 1.

Erythromycin is a member of the macrolide group of antibiotics. Its chemical structure (Fig. 1) offers no ready clue to its mechanism of action at the molecular level with the possible exception that there is a general structural relationship to other antibiotics containing amino sugar, such as streptomycin.

Two observations, however, may offer leads to the elucidation of the mechanism of action of erythromycin: (i) erythromycin has been found by Vasquez (*8*) to inhibit the interaction between radioactive chloramphenicol and sedimented ribosomal fractions of bacteria; we have readily confirmed this observation and have also shown that this inhibition is competitive in nature (*9*); and (ii) we have also recently observed that erythromycin is capable

Table 1. Inhibition by erythromycin (E) of polyphenylalanine synthesis in a cell-free ribosomal system from *E. coli*, strain Gratia. Erythromycin was used as the lactobionate derivative. The complete reaction system contained the following additions in a volume of 0.27 ml; in micromoles: adenosine triphosphate (ATP) (di-K salt), 2.0; guanosine triphosphate (GTP), 0.2; phosphoenolpyruvate (PEP) 1.2, tris 2.0 (*p*H 7.8); KCl 40.0; magnesium acetate, 6.0; mercaptoethanol, 29.0; in millimicromoles: L-phenylalanine C^{14}, 0.5, with a specific activity of 297 mc/mmole; in micrograms: transfer-RNA (*10*), 400; polyuridylic acid (polyU) [K salt, (*11*)], 25; the extract consisting both of ribosomes and enzymes contained 232 μg of bacterial RNA. The mixture was incubated for 20 minutes at 37°C, 5.0 ml of 10 percent trichloracetic acid were then added, the mixtures were heated at 95°C for 20 minutes, and the precipitates formed were washed with 1 percent trichloracetic acid by centrifugation until there was no detectable radioactivity in the washings. Finally the precipitates were collected on Millipore HA filters and the radioactivity was determined with a Nuclear-Chicago liquid scintillation counter. CS, complete system.

Additions	Radioactivity in total ppt. (counts/min)
CS	33,649
CS − ATP	2,762
CS − polyU	764
CS + 1.0 μmole E	14,815
CS + 1.5 μmole E	6,765

of precipitating RNA and certain synthetic polyribonucleotides (*9*). We infer that the mechanism of action of erythromycin may be related to that of chloramphenicol and may involve an interaction with one or several categories of RNA that mediate protein biosynthesis.

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Membrane Properties of Barnacle Muscle Fiber

Abstract. *Membrane properties of the giant muscle fiber of the barnacle* Balanus nubilus *were studied by controlling the ionic composition of the external and internal media. The resting potential decreases with increasing external K-concentration,* $[K^+]_{out}$, *and decreasing internal K-concentration,* $[K^+]_{in}$, *over a considerable range. Spike potentials are elicited when the internal calcium ions are removed, and the overshoot is determined by the ratio between* $[Ca^{++}]_{out}$ *and* $[K^+]_{in}$ *and not by the external or internal* $[Na^+]$.

Giant muscle fibers 1 to 2 mm in diameter have been described recently in the barnacle *Balanus nubilus* by Hoyle and Smyth (*1*). This preparation offers an excellent opportunity for studying membrane physiology by controlling the internal ionic composition of the fiber.

A resting potential of 70 to 80 mv was found with a 3*M* KCl-filled glass micropipette. Depolarization produced by an outward current through a separate micropipette even well above the threshold for mechanical response did not elicit a spike but gave only a graded potential, as is usual for crustacean muscle. The membrane became capable of producing an all-or-none spike when the Ca^{++} concentration inside the fiber was reduced. The internal solution was changed by the following two methods.

1) In the intracellular injection method, a single muscle fiber was isolated with a tendon on one end, and the other end was cut after it had been tied with a thread. With the fiber mounted horizontally on a glass plate, a glass injection pipette 0.2 to 0.5 mm in diameter was introduced longitudinally through a small incision made close to the cut end of the fiber (see diagram *A*, Fig. 1). The test solution was injected while the pipette was being advanced so that the solution was distributed over the whole length of the fiber up to the tendinous end. The injection was continued until the diameter of the fiber became 1.5 to 2.0 times the original diameter. This would presumably make the internal ionic composition of the treated fiber close to that of the injecting solution. The fiber apparently tolerates this inflation rather well. The first 5 mm from the incision was covered with paraffin oil. The rest of the fiber (1.0 to 1.5 cm) was immersed in barnacle physiological saline (*1*); the length of this portion was usually two to three times the space constant of the fiber. The membrane potential change was recorded with a micropipette close to the tendinous end while polarizing currents were applied through the injection pipette (Fig. 1*A*). When a Ca^{++}-binding agent was injected, such as K_2SO_4 solution with K salt of EDTA (ethylenedinitrilotetraacetic acid) or K salt of citrate solution ($[K^+] = 490$ m*M*, the osmotic pressure maintained by adding sucrose; and *p*H adjusted to 6.9), the membrane became capable of initiating an all-or-none spike as shown by Fig. 1, *C1* and *C*2.

2) In the sandwich method, a piece of membrane was used instead of a whole muscle fiber. First a Ca^{++}-binding agent was injected into an isolated muscle fiber as in the first method. Then the middle 1 cm of a single muscle fiber was isolated by cutting away both ends. This length of muscle fiber was opened by cutting the membrane longitudinally along one side. The opened membrane was placed between two thin glass plates in each of which a hole of about 1-mm diameter had been drilled. The inner side of each plate had been covered with vaseline to avoid leakage, and the two plates were adjusted so that the holes were superimposed (see diagram *B*, Fig. 1). Figure 1*F* shows the spike potentials that could be obtained when a K_2SO_4 solution with 10 to 20 m*M* K salt of EDTA was in contact with the internal side of the membrane. The solution on the external side contained 169 m*M* Ca in this case. This second method is advantageous when the observation of effects of different internal solutions is desired for the same fiber membrane, although a slow deterioration was usually found in such preparation. At the present stage it is not possible to remove the entire internal content of the fiber from the membrane, and so it is difficult to make the ionic concentration just inside the membrane identical to that of the test solution, even by the sandwich method. Most of the results described below were obtained by the first method.

The resting potential of the muscle fiber changed linearly with the logarithm of $[K^+]$ in the external saline, with a slope of 56 mv for a tenfold increase of $[K^+]_{out}$. The resting potential changed its sign from the negative