ing will be performed under the arbitrary assumption that it is due strictly to stimulation of a broad region on the basilar membrane, in accordance with a "place" theory of hearing. The calculated informational capacity depends only on the masking and intensity data, and is independent of which auditory mechanism is used.

On this basis, response of the ear at any time can be described by the "stimulation profile," a graph of stimulus intensity vs. position on the basilar membrane. It represents a sort of short period Fourier analysis of the sound along the membrane. The effect of the phenomenon which leads to masking of adjacent frequencies can be represented by stating that the derivative of the profile cannot exceed a certain value. Thus intense sounds "swallow up" faint ones at neighboring frequencies, because stimulus intensity decays insufficiently with frequency to allow the weak tone to be perceived.

At any point in the hearing region, a certain length along the basilar membrane will have to be traversed before a single intensity j.n.d. can be perceived. This can be called the masking distance, and is larger for low tones than for high ones, in general. With simplifying assumptions, the total number of distinguishable configurations possible to the stimulus profile can be counted. Using the Riesz intensity perception data (*3*), and averaging with the Wegel and Lane masking data (*6*), a length of about 0.04 mm on the basilar membrane is obtained as the average masking distance.

Any of three relations may be observed between two points separated by this distance on the profile. The point at higher frequency may be (1) the same intensity, (2) one j.n.d. more intense, or (3) one j.n.d. less intense than the lower frequency point. A simple calculation gives approximately $230 \times 3^{(32/.04)}$ as the total possible number of profiles, since there are 230 j.n.d.'s in the average intensity range, three choices of profile direction in one masking distance, and 32/.04 or 800 masking distances along the total basilar membrane. Hence, the total number of bits per profile is $\log_2 (230 \times 3^{800})$, or about 1,300 bits. Dividing by 1/6 sec, the time required for the intensity discrimination measurements, a figure of about 8,000 bits/sec is obtained for the informational capacity of one human ear.

Two assumptions cause this figure to be crude. First, it is likely that the use of intensity measurements of shorter duration than 1/6 sec would increase this figure, by allowing more profiles per sec to be possible, even though the amount of information per profile would decrease somewhat. Second, the use of a single averaged masking distance is subject to some error. Phase information, generally of no value except to binaural phenomena, is also neglected.

An important observation is that the dynamic range of the ear scarcely affects the final calculations. Only when the intensity range of the intelligence is cut down to 10 or 20 db is a sufficient fraction of the possible profiles ruled out to lower appreciably the informational capacity.

Since about 29,000 ganglion cells (*2*) are to be found in a cochlear nerve, we observe about 0.3 bit/sec of information per nerve fiber. This low figure naturally results from the lack of coding in the cochlear innervation. If the capacity is taken as 1.3×10^6 distinguishable tones/sec, then about 40 tones/sec can be accounted for by each fiber. This is about 10% of the maximum number of impulses which an adapted nerve fiber can carry.

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The Effect of Aureomycin on *Endamoeba histolytica* in Vitro

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Antibiotics obtained from molds or bacteria have shown thus far a very limited range of usefulness in diseases caused by protozoan parasites, with the exception of amebiasis. Good clinical results have been obtained in human infections with *Endamoeba histolytica* by the administration of penicillin (*1*), bacitracin (*6*), and aureomycin (*5, 6*), although it is inferred by some that improvement is due to the action of the antibiotics upon secondary bacterial invaders rather than directly on the amebas (*4, 6*). In the case of penicillin this seems to be substantiated by results obtained *in vitro*, in that no direct effect upon amebas was observed with levels as high as 30 Oxford units per ml for 48 hr (*4*). Gramicidin and subtilin were reported to be effective *in vitro*, and it was stated that subtilin lowers surface tension, causing rupture of the membrane of *E. histolytica* (*2*). In the course of a screening program *in vitro* for amebacidal substances in our laboratories, it was found necessary to evaluate the effects of antibiotics by a somewhat different procedure from that commonly used, in order to separate effects produced on amebas from bactericidal action against associated bacterial flora. These studies revealed that aureomycin produces what appears to be a direct effect on trophozoites of the strain of *E. histolytica* used.

The strain of *E. histolytica* used in this study was obtained through the courtesy of Charles Rees of the National Institutes of Health. It was designated as the NRSta strain, and when first received was growing in association with a single species of bacterium. Since a mixed bacterial flora gave more profuse growth, however, the culture was eventually maintained with a mixed unidentified bacterial flora for bioassays. Liver infusion agar (Difco) was used as a substrate, with either a Loeffler's-Ringer's or horse serum-saline overlay and a

TABLE 1

EFFECT OF AUREOMYCIN HCl AND EMETINE HCl ON *Endamoeba histolytica* IN VITRO, USING ASSAY METHOD NO. 1

Dilution	Aureomycin HCl								Emetine HCl		
	Assay No.								Assay No.		
	1	2			3			4	1	2	
		Tube 1	Tube 2	Tube 3	Tube 1	Tube 2	Tube 1	Tube 2		Tube 1	Tube 2	Tube 3
1:100	0						0	0	0			
1:1,000	+						0	0	0			
1:5,000	+						0	0	0			
1:10,000	+	0	+	+	0	0	0	0	0	0	0	0
1:20,000		+	+	+						0	0	+
1:30,000		+	+	+						+	+	+
1:40,000		+	+	+						+	+	+
1:50,000	+	0	+	0	+	+	0	0	+	+	++	+++
1:60,000		+	+	+						+	++	++
1:70,000		+	+	+						+	++	++++
1:80,000		0	0	+						+	0	+
1:90,000										+	+++	+
1:100,000	+++	0	+	0	++	+	0	+	+++	++++	++	++
1:1,000,000 ...	++++						++	++	++++			

Controls—++, +++, or ++++ throughout.
0 = No amebas found.
+ = Scanty growth of amebas.
++, +++, ++++ = Moderate to heavy growth of amebas.

small quantity of rice starch. The Loeffler's-Ringer's overlay was prepared by dissolving 250 mg of Loeffler's dehydrated blood serum in 1,000 ml of sterile Ringer's solution. The horse serum-saline overlay was a mixture of 1 part fresh sterile horse serum in 7 parts of 0.85% sterile NaCl solution. Subcultures were made at intervals of 48 hr. Two methods were used for bioassay:

Method 1—Substances for test were dissolved in the overlay at varying dilutions, and subcultures of amebas were made to liver infusion agar slants covered with the test mixture. Usually, duplicate or triplicate tubes of various dilutions of the test mixture were inoculated. Approximately 0.1 ml of inoculum containing many motile amebas, rice starch, and bacteria from a 48-hr culture was used for each subculture. Incubation was carried out at 37.5° C for 48 hr, and the cultures were examined microscopically at the end of this period. Activity was expressed as shown in the accompanying tables. Since this method does not eliminate effects produced against the associated bacterial flora, any clearing of bacteria produced in the cultures was noted at the end of the 48-hr period, particularly if amebas were scarce or absent.

Method 2—Overlay containing many motile amebas, rice starch, and bacteria was pooled from 48-hr cultures, and 2 ml of this pooled material was incubated in 8 ml of physiological saline to which the substance to be tested had been added. At the end of the exposure time (10–60 min), the mixture was centrifuged at 2,000 rpm for 3 min, decanted, and washed with physiological saline. Additional centrifugation and washing were carried out three or four times. After the last wash and centrifugation, the sediment, containing amebas and granules of rice starch present in the original overlay, was introduced into culture media which had been seeded with the mixed bacterial flora used routinely in subcultures. Amebas for control subcultures were treated in exactly the same way with physiological saline to which no test substance had been added. Growth of amebas was then recorded at the end of 48-hr incubation. There is, of course, the possibility that small amounts of test substances might be retained by the amebas during centrifugation and washing, or could be adsorbed on the granules of rice starch present in the pooled overlay. However, in every case in which this method was used, bacterial growth was as profuse in bacterial-seeded cultures inoculated with treated amebas as in controls, suggesting that very little of the test substance was carried over into the subcultures.

Table 1 presents data on the effects of aureomycin HCl and emetine HCl in varying dilutions and in different assays on the NRSta strain of *E. histolytica*, using assay Method 1. Growth of amebas was scarce or prevented completely in dilutions of aureomycin as high as 1 : 100,000 in some assays. Variations occurred in different assays, but these were not as great as those exhibited in dilutions of emetine beyond 1 : 40,000. Bacterial growth was considerably inhibited in higher dilutions of aureomycin, and a complete clearing of bacteria was observed in low dilutions, whereas bacterial growth in emetine dilutions, as determined by turbidity, was the same throughout. The fact that bacterial growth was inhibited or prevented completely in cultures containing aureomycin makes it difficult to determine whether a direct effect was produced on the amebas when this assay method was used.

The data given in Table 2, using assay method No. 2, demonstrate that a very pronounced effect against the amebas was produced by aureomycin in a dilution of 1 : 1,000, with from 10- to 60-min contact. Less pronounced effects were produced by dilutions of 1 : 10,000,

TABLE 2

EFFECT OF AUREOMYCIN HCL AND EMETINE HCL ON *Endamoeba histolytica in Vitro*, USING ASSAY METHOD NO. 2*

Compound	Dilution	Growth on liver infusion agar in 48 hr								
		After 10-min contact			After 30-min contact			After 60-min contact		
		Tube 1	Tube 2	Tube 3	Tube 1	Tube 2	Tube 3	Tube 1	Tube 2	Tube 3
Aureomycin HCl	1 : 1,000	0	0	0	0	0	0	0	0	0
"	1 : 10,000	++	+	++	+	+	+	0	+	0
"	1 : 100,000	+	++	+	++	+	++	++	+++	+
Controls	(physiological saline)	+++	++	++++	+++	++++	++	+++	++	++
Aureomycin HCl	1 : 1,000							0	0	0
Emetine HCl	1 : 1,000							+++	+++	+++
Controls	(physiological saline)							+++	+++	+++
Aureomycin HCl	1 : 1,000							+	+	+
"	1 : 10,000							++	++	++
"	1 : 100,000							++	+++	+++
Emetine HCl	1 : 1,000							+++	+++	+++
"	1 : 10,000							+++	+++	+++
"	1 : 100,000							+++	+++	+++
Controls	(physiological saline)							++++	++++	++++

* Density of growth recorded as in Table 1.

although in the first assay listed the effect at this dilution was measurably greater after 60-min contact than after 10-min contact. Emetine, on the other hand, showed no evidence of direct amebacidal action after contact periods of 60 min at dilutions of 1 : 1,000.

The effect of aureomycin in dilutions of 1 : 1,000 at different pH ranges is shown in Table 3, using assay method No. 2. No measurable difference occurred in the effects produced within the pH range used.

TABLE 3

EFFECT OF AUREOMYCIN HCL AT DIFFERENT PH RANGES ON *Endamoeba histolytica* IN VITRO, USING ASSAY METHOD NO. 2

Compound	pH			Growth on liver infusion agar 48 hr after contact	
	Contact solution	Supernatant after first centrifugation	Overlay of liver infusion agar	Tube 1	Tube 2
Aureomycin HCl* ..	3.12	5.29	6.69	0	0
" ..	6.85	6.51	6.69	0	0
" ..	8.00	6.73	6.69	0	0
Controls ..... (physiological saline)	6.41	6.70	6.69	++	+++

* In 1 : 1,000 dilution. Contact period was 60 min for aureomycin and controls.

Although evidence of the direct effect *in vitro* of aureomycin against *E. histolytica* obtained in these studies does not signify that a similar mode of action is involved in the disappearance of trophozoites in human infections treated with aureomycin, this possibility must be considered. Similar assays *in vitro* have been conducted using streptomycin, bacitracin, and chloramphenicol, and none of these antibiotics in 1 : 1,000 dilutions have shown measurable direct effects upon *E. histolytica* when left in contact with motile amebas for 60 min. Neither emetine HCl nor chiniofon in dilutions of 1: 1,000 killed the amebas during this period of contact.

It has been stated previously that little activity has been demonstrated *in vivo* by antibiotics against protozoan parasites. Tyrothrycin (*7*) and clavacin (*3*) show antimalarial activity in birds, but the dosage is close to the toxic level. We have found aureomycin to be effective against *Plasmodium lophurae* Coggeshall in ducks, using intravenous doses of from 25 to 100 mg/kg twice daily for seven doses. All of the plasmodia are not removed within this dosage range, but reductions as great as 90% (at a dosage of 100 mg/kg), with pronounced degenerative changes in all stages of the asexual cycle, were noted. Streptomycin, bacitracin, or penicillin produced no measurable effects against *Plasmodium lophurae* within this dosage range. A direct analogy cannot be drawn between the antimalarial effect of aureomycin *in vivo* and its activity either *in vivo* or *in vitro* against *E. histolytica*. It is again apparent, however, that attempted explanations for the mode of action of aureomycin in clinical amebiasis must include the possibility that the amebas, as well as the intestinal flora, may be affected *in vivo*, since this antibiotic does possess protozoacidal properties.

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Precipitation of Menstrual Bleeding in Monkeys by a Folic Acid Antagonist[1]

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Failure of estrogens to induce growth of the female reproductive tract in the absence of folic acid was first described for the chick (*4*). Similar results have been obtained with the frog (*2*), the rat (*5*), and the monkey (*3*). In 1948, Hertz (*3*) reported that six out of eight sexually immature monkeys on a folic acid-free diet failed to show cornification of the vaginal mucosa, development of the external genitalia, or growth of the sex skin after treatment with estradiol benzoate. Data presented in this report show that maintenance of the uterine endometrium of monkeys by estradiol[3] is also affected by a lack of folic acid.

Five castrated female monkeys (*Macaca mulatta*) were injected subcutaneously with 10 μg of estradiol daily throughout the experiment. This dosage of estrogen is sufficient to maintain the endometrium indefinitely without bleeding. After 20 days' pretreatment with estradiol, the monkeys were given daily injections of a folic acid antagonist, aminopterin,[4] along with the estrogen. The susceptibility of monkeys to a lack of folic acid and individual differences of tolerance to aminopterin made it advisable to start the treatment with a low dose of the antagonist and to increase the amount, about every 10 days, until the animals menstruated or were prostrate due to the vitamin deficiency. This schedule was adhered to for all the animals, with two exceptions. Monkey 181 was kept on 100 μg of aminopterin throughout the experiment, and monkey 175 was continued on 500 μg of aminopterin when that level was reached (Table 1).

All monkeys were examined daily for menstrual bleeding, and blood counts were made at frequent intervals. Complete post-mortem examinations were performed on the animals when menstruation was noted or when the animals were in a state of collapse and near death.

[1] Aided in part by a grant from the American Cancer Society, Committee on Growth of the National Research Council.

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[3] Estradiol was obtained through the courtesy of Schering Corporation, Bloomfield, New Jersey.

[4] Aminopterin (4-aminopteroylglutamic acid) was obtained through the courtesy of Dr. E. L. R. Stokstad, Lederle Laboratories, Pearl River, New York.

Menstruation occurred in three of the five monkeys treated with estradiol and aminopterin (Table 1). These animals had received the antagonist for a total of 23–48 days, while the two monkeys that failed to menstruate had been under treatment for 22 days. It is apparent that neither length of treatment nor amount of antagonist given can explain the failure to produce menstruation in these two monkeys. A possible explanation may be found in the extreme variability in length of time required to produce a folic acid deficiency in monkeys (*1*), and the difficulty in keeping them alive once the deficient state is attained. Apparently, in order to produce menstruation in monkeys during estrogen treatment, it is not only necessary to produce a folic acid-deficient condition, but it is also necessary to keep the animals alive long enough for menstruation to occur.

TABLE 1

PRECIPITATION OF MENSTRUATION BY AMINOPTERIN IN MONKEYS UNDER DAILY TREATMENT WITH ESTRADIOL*

Monkey No.	Body wt in kg	Days of treatment with aminopterin				Total No. days of treatment	Results
		100 μg	200 μg	500 μg	1 mg		
169	4.52	10	10	9	9	38	Menstruation
175	3.36	10	10	28	—	48	Menstruation
179	3.77	10	10	2	—	22	No menstruation
180	3.69	10	12	—	—	22	No menstruation
181	3.52	23	—	—	—	23	Menstruation

* Castrated monkeys under daily treatment with 10 μg of estradiol alone for several months to a year failed to menstruate during the course of treatment.

Previous work in this laboratory has shown that the daily dose of estradiol used in these experiments will induce maximal growth of the endometrium in monkeys within 20–30 days, and that menstruation does not occur when such treatment is continued for several months. Therefore, our results indicate that in the absence of folic acid, estrogen cannot maintain the uterine endometrium or prevent menstrual bleeding.

Menstruation failed to occur in three castrated monkeys that were first given 10 μg of estradiol for 20 days and continued on daily injections of 10 μg of estradiol, 2 mg of progesterone and aminopterin. Also, three castrated animals given 2 mg of progesterone and aminopterin daily, following a 20-day pretreatment with estrogen, did not menstruate. Thus it would appear that estrogen and progesterone act through different metabolic systems and only estrogen is inhibited sufficiently to precipitate menstruation.

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