it is certain that the principal cause for the decrease in osmotic pressure is the decrease in the concentration of serum protein.

Perhaps the most dangerous error in Kesselman's paper is the implication that the osmotic pressure at one pH can be calculated from that at another pH by the use of the Donnan equation *for ideal electrolytes*. It has been shown (*2, 4*) that the variation of pressure with pH for albumin is less than one third the value indicated by this equation, so that it is actually more accurate to assume that the variation is zero than to use this equation.

It is true that the osmotic pressure of normal serum under physiological conditions may be calculated by Kesselman's equations, but it may be measured more easily than the quantities from which he calculates it.

The plasma proteins cannot be characterized merely by the albumin-globulin ratio. Oncley, Scatchard and Brown (*6*) report the physical properties of ten of the globulins of normal human plasma, with contributions to the osmotic pressure varying from 5% to more than 75% that of albumin. In pathological sera the quantity of each of these does not change in the same ratio, so the character of the globulin changes as well as its quantity.

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Fumagillin (H-3), a New Antibiotic with Amebicidal Properties[1, 2, 3]

Max C. McCowen, Maurice E. Callender, and John F. Lawlis, Jr.

The Lilly Research Laboratories, Indianapolis, Indiana

A new crystalline antibiotic, designated as H-3, with antiphage activity, has been isolated from cultures of a species of *Aspergillus* sp. Hanson and Eble (*1*) first isolated the active concentrates that were capable of inhibiting *Staphylococcus aureus* 209 bacteriophage.

Little antibacterial and antifungal activity was demonstrated, and no antiviral activity was exhibited *in vivo* when tested against MM virus and influenza (PR8A) infections in mice.

We have found this antibiotic to be an extremely potent amebicide, producing inhibitory effects *in vitro* against a strain of *Endamoeba histolytica* (NIH200) with mixed bacterial flora at dilutions as high as 1:131,072,000. The present paper reports preliminary tests designed to investigate the amebicidal potentialities of this antibiotic.

The initial tests showed a crude concentrate (Lot 109-TEE-1) to be effective at dilutions of 1:8,192,000 to 1:16,384,000 in cultures of *E. histolytica* with mixed bacterial flora as shown in Table 1.

TABLE 1

EFFECT OF H-3 ON *E. histolytica* (NIH200) *in Vitro*

Preparation No.	Multiplying bacteria	Minimum effective dilution*
109-TEE-1	Present†	1: 8,192,000
	"	1: 16,384,000
	Absent‡	1: 4,096,000
41-TEE-4 (crystalline)	Present	1: 131,072,000
77-TEE-4 (")	"	1: 65,536,000

* Control tubes with untreated amebae gave heavy growth at 37.5° C for 48 hr.
† Balamuth egg yolk infusion media.
‡ Shaffer-Frye media.

This crude concentrate of the antibiotic when tested in cultures of *E. histolytica* in the absence of multiplying bacteria modified (*2*) after the technique described by Shaffer *et al.* (*3*) was effective at dilutions of 1:4,096,000. Since no associated bacterial growth influenced the growth of the amebae in these cultures, the activity of antibiotic H-3 is interpreted as being direct upon the amebae. H-3 is the first antibiotic or amebicide that has demonstrated such effective amebicidal properties in our laboratories.

Two lots of crystalline H-3 (41-TEE-4 and 77-TEE-4), reported to be five times more active against *S. aureus* phage than the crude concentrate, were approximately as effective against *E. histolytica* and demonstrated activity at dilutions of 1:131,062,000.

The amebicidal properties of H-3 were further tested *in vivo* using young rats experimentally infected with cysts of *E. histolytica* (NIH200) (*4*). The crude concentrate (Lot 109-TEE-1) was found to clear rats of cecal infections of *E. histolytica* when four divided doses were administered orally for 2 days. The total dosage was 36 mg/kg as shown in Table 2. Crystalline preparations (Lots 41-TEE-4 and 77-TEE-4) cleared rats of amebae when four divided doses were administered orally for 2 days. The total dosage was approximately 11 mg/kg. No antibacterial activity has been observed against the intestinal flora in rats.

Further tests were made using the crystalline antibiotic H-3 (Lot 77-TEE-4) against experimental amebiasis in young rabbits infected by the technique described by Tobie (*5*). The antibiotic was found to clear the animals of *E. histolytica* when 4 divided doses were administered orally for 2 days as shown in Table 2. A total dosage of 100 mg/kg was given during this period of treatment.

Additional tests are being conducted in experimental animals to establish an amebicidal end point.

[1] A preliminary report.
[2] Fumagillin, generic name given antibiotic H-3 by the Upjohn Company.

[3] We wish to thank the Upjohn Company which generously supplied us with samples of crude and crystalline H-3.

TABLE 2

EFFECT OF H-3 ON *E. histolytica* (NIH200) INFECTIONS IN YOUNG RATS AND RABBITS

Preparation	Dosage	Mg/kg	Cleared of *E. histolytica* Animals infected
Young rats, 30–35 g			
109-TEE-1	1.75 Mg×4	210	7/ 7
	0.625 " "	71.25	4/ 4
	0.375 " "	36	7/ 7
	0.1 " "	8.8	0/ 7
Infected controls	—	—	1/28
41-TEE-4 (crystalline)	0.1 Mg×4	11.2	4/ 4
	0.05 " "	8.0	0/ 5
Infected controls	—	—	0/ 8
77-TEE-4 (crystalline)	0.1 Mg×4	11.2	4/ 4
	0.05 " "	5.6	1/ 5
Infected controls	—	—	0/ 7
Young rabbits, 10–12 wks old			
77-TEE-4 (crystalline)	30.5 Mg×4	100	1/ 1
	41.5 " "	100	1/ 1
Infected controls	—	—	0/ 3

H-3 was found to be ineffective *in vivo* when tested against *Trypanosoma equiperdum, T. gambiense,* and *Spirochaeta novyi* infections in mice.

From the present experimental data this new antibiotic H-3 should possibly be one of the best direct-acting amebicides.

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Preparations for Electron Microscopy of Residual Chromosomes[1, 2]

A. R. T. Denues[3]

Detroit Institute of Cancer Research, Detroit, Michigan

Both the interest in means for preparing chromosomal material for electron microscopy and the undeveloped status of such direct studies of the fine structure of chromosomes are attested by the recent publication of a number of technical papers. The place of special techniques in this work is indicated by the relative opacity in the electron beam of whole cells (*1, 2*), of nuclei (*3, 4*), and even of isolated whole chromosomes (*5, 6*). Microdissection (*7, 8*), thin sectioning (*9*), squashing with use of replicas (*10*), and smearing (*11*) have been used. Another approach has been through isolation procedures involving physical reductions of cells and fractional centrifugations following Claude and Potter (*12*), Pollister and Mirsky (*13*), and Mirsky and Ris (*14*). Few studies of such isolated material have been made with the electron microscope, some without (*15*) and some with (*6, 16*) use of molar NaCl as developed by Mirsky and Ris (*17*) for the preparation of "residual chromosomes."

This note offers a brief description and some preliminary results of such a technique employing reiterated extractions of the isolated chromosomes with *M* NaCl, ultraviolet absorption spectrophotometry to assay the extractions, and other controls. Preparations of this kind have been the subject of extensive (*17*) and specific (*18*) chemical studies, but in earlier electron microscopy of such material (*16*) only two extractions of the chromosomes with *M* NaCl were made.

In a laboratory at a temperature near 0° C, erythrocytes were separated by centrifugation at 600 *g* from chilled whole blood of White Rock chickens. The supernatant and buffy coat were decanted, and any extraneous sediment was discarded. The cells were repeatedly washed with Gey's solution less the sugar (*19*) (hereafter referred to as "Gey's salt solution") until examination in the light microscope disclosed homogeneous preparations of apparently normal red cells. Suspensions of these cells in Gey's salt solution were reduced in a Waring Blendor by a series of full-speed runs, each of 1 min duration, followed by a cooling period, until microscopic examination revealed strands comparatively free of unbroken nuclei. The total time of treatment in various experiments ranged from 5 to 15 min. A series of centrifugations at 600 *g* then followed, with decantation, gentle resuspension in fresh Gey's salt solution, and pouring to clean tubes at each step, until microscopic examination, including use of Darlington's aceto-orcein, disclosed chromosome threads substantially free of extraneous material. Residual chromosomes were obtained by forcibly injecting a suspension of this material in Gey's salt solution through a #27 hypodermic needle into an excess of *M* NaCl buffered to about pH 7 with *M*/150 Sørensen's phosphate; the mixture was vigorously shaken after addition of each increment of suspension. The homogeneous viscous dispersion resulting was centrifuged for 1–4 hr at about 18,000 *g*, the supernatant being decanted for absorption analysis. Part of the sediment was fixed and mounted for electron microscopy, the bulk being again extracted with fresh *M* NaCl. This extraction procedure was repeated up to 12 times in some experiments. Specimens were mounted on collodion films and shadowed with chromium by standard techniques and examined in an RCA type EMU-2 instrument fitted with a bias gun.

Absorption spectra in the range 2,100–3,200A confirmed the existence of maximum absorption near 2,600A for the supernatants from first and second

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[3] Now at the Sloan-Kettering Institute for Cancer Research, New York City.