Summary and discussion. The experiments which have been described demonstrate the capacity of the Lansing and Brunhilde strains of poliomyelitis virus to cause cell injury and death. This cytopathogenic property is revealed (1) by degenerative changes produced in infected tissue fragments in flask cultures and apparent on histologic examination; (2) by failure of such tissue fragments to exhibit normal cell migration when explanted to plasma cultures; (3) by degeneration of newly emigrated cells in roller-tube cultures; (4) by decreased acid production by infected cells. The conclusion that certain of these manifestations of injury are induced as a result of infection by the virus is further supported by the fact that type specific immune serum prevents their development. These phenomena are of interest from two general points of view. First, they leave no doubt that poliomyelitis virus *in vitro* can multiply in cells other than those of the nervous system and cause profound injury of such cells. Secondly, they provide criteria by which the presence of the virus can be recognized *in vitro* and hence may afford a basis of technics for isolating virus from patients or animals, for the quantitative assay of virus, for serologic typing and possibly for the screening of chemotherapeutic and antibiotic substances. Further study will be required of the reliability and practicability of the application of these phenomena to such ends.†

† The authors are grateful to Marguerite Buckingham, Gloria Florentino, Carolyn Keane, and Sheila Richardson for essential technical assistance and to Dr. Duncan Reid and the members of the staff of the Boston Lying-in Hospital for their assistance in obtaining materials for this study.

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Biological Studies on *Entamoeba histolytica*. IV. Direct Action of the Antibiotic, Prodigiosin.* (18203)

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Antibiotics have been applied against *Entamoeba histolytica* as part of the search for more effective chemotherapeutic agents. Although various accepted antibiotics (penicillin, streptomycin, aureomycin, chloromycetin, etc.) have been tested *in vitro*, no clear-cut evidence has been presented of their direct action upon the amoebae. A recent report from Anderson's laboratory aptly summarizes the situation: "All antibiotics tested to date have shown some degree of bacteriostatic activity, and death of the ameba in cotton-stoppered tubes is shown to be due to inhibition of growth of the associated bacteria rather than to direct action of the antibiotic on *E. histolytica*"(1). The present experiments were designed to meet these objections, and the data presented below are submitted as the first proof of direct action by an antibiotic on *E. histolytica*.

Our original interest in prodigiosin sprang both from the long-known antagonism exhibited by chromogenic strains of *Serratia marcescens* against various microorganisms, and the feasibility of using the corresponding antibiotic pigment, prodigiosin, whose extraction and structure had been elucidated by Wrede and co-workers(2). Antibacterial activity has been demonstrated against gram-positive types, including *Bacillus anthracis* (3), *Corynebacterium diphtheriae* and *Neisseria gonorrhoeae*(4), *Pasteurella pestis*(5),

* Part of a project supported by a research grant from the National Institutes of Health, Public Health Service.

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3. Rettger, L., *J. Infect. Dis.*, 1905, v2, 562.

4. Eisler, M., and Jacobsohn, I., *Z. f. Hyg. Infektionskr.*, 1936, v117, 76.

B. anthracis and staphylococci(6). Hettche claimed that *B. anthracis* was immediately killed by 1:50,000 prodigiosin, one-half the concentration required for staphylococci, while gram-negative "colon and paratyphoid" bacilli were unaffected. Free-living protozoa have been reported susceptible to chromogenic strains of *S. marcescens: Glaucoma, Colpoda, Paramecium*(7,8), and soil amoebae (9). Fischl(10) demonstrated that prodigiosin perchlorate was trypanocidal in mice infected with *T. brucei.* The present authors (11) presented their partial data before the American Society of Parasitologists. The only report of fungicidal activity is that of Lack (12), who found that *Coccidioides immitis* was destroyed *in vitro* after 48 hours' exposure to 1:100,000 prodigiosin.

Materials and methods. Prodigiosin was extracted in this laboratory from surface cultures of *S. marcescens* grown on Hayes' medium(13) adjusted to pH 6.7 and containing 1.5% agar. The cultures were harvested after four days' incubation in the dark at 23-25°C, and the pigment was extracted according to the method of Wrede and Hettche(14). The properties of our compound agreed with those of the original, which has been identified as a tripyrrole methane bearing the formula $C_{20}H_{25}ON_3$. As a further check the absorption characteristics were measured with a Beckman quartz spectrophotometer, model DU.† The curves (Fig. 1) conformed strikingly to those of Ehrismann and Noethling (15), although there was an apparent typographical error in the value accorded the ordinate in their paper.

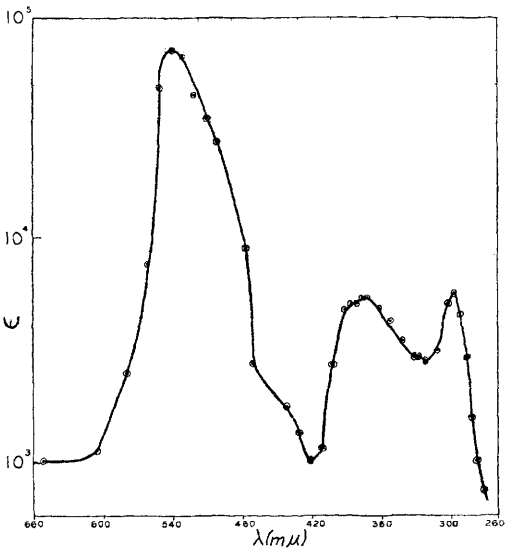

FIG. 1.
Absorption curve of prodigiosin. Extinction coefficient (ε) plotted against wavelength (mμ).

Two distinct strains of *E. histolytica* were employed: the *UC* strain associated with a mixed bacterial flora, and the *NRS* strain associated only with *Aerobacter aerogenes.* The test medium was the same buffered egg-yolk infusion (pH 7.40) plus rice starch in which stocks have been maintained for several years(16). In different experiments culture tubes contained either 10 ml or 30 ml medium, the former receiving 0.5 ml inoculum, the latter 1.0 ml. Bacterial populations were measured by decimal-dilution plating, counts being made of the last two dilutions. Amoebic inocula were counted on hemocytometers and exceeded 100,000 in all critical experiments; yields were usually recorded after 48-72 hours as simply present or absent. In all apparently negative tubes, bacterial counts were made and 0.2 ml sediment

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† These measurements were made under the direction of Dr. I. M. Klotz of the Department of Chemistry.

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TABLE I. Effects of 48-hr Exposure to Prodigiosin in Monobacterial Cultures of *Entamoeba histolytica* (*NRS* strain with *Aerobacter aerogenes*.)

Features observed	Concentrations of prodigiosin					
	Control	1:2 × 10^6	1:10^6	1:8 × 10^5	1:4 × 10^5	1:2 × 10^5
Survival of amoebae*	+	+	+	+	—	—
Bacteria (× 10^6 per ml)	780	950				1100
Oxidation-reduction potential (E_h in mv.)	—185	—185	—220		—240	—210
pH	7.08	7.10	7.03	7.05	7.04	7.20

* The same amoebacidal endpoint was obtained with inocula varying between 17,000 and 251,000 amoebae.

TABLE II. Effects of 48-hr Exposure to Prodigiosin in Cultures of *Entamoeba histolytica* (*UC* strain with Mixed Flora.)

Features observed	Inocula	Concentrations of prodigiosin				
		Control	1:2 × 10^5		——1:10^5——	
Survival of amoebae	102,000	+	—		—	
	391,000			+		—
Bacteria (× 10^6 per ml)	7.3	445	351		213	
	27.9			307		264
Oxidation-reduction potential (E_h in mv.)		—280	—217		—186	
				—208		—222
pH		6.99	7.14		7.15	
				7.09		7.12

was subcultured into sterile medium for an additional 72 hours' incubation. Oxidation-reduction potentials (E_h) and pH were measured with a model G Beckman meter; for the former, duplicate gold-plated platinum electrodes were inserted permanently in some series of tubes. Stock solutions of prodigiosin were maintained in ethanol and checked for sterility before use. Dilutions were prepared in the range 1:20,000,000 to 1:100,000, and controls received an equivalent concentration of ethanol (max. 0.75%).

Results. Tables I and II summarize for the two strains respectively the major effects on viability of amoebae and associated bacteria, as well as changes in E_h and pH. Amoebae in the monobacterial culture proved more susceptible to the action of prodigiosin, even in the absence of demonstrable action against *A. aerogenes*.‡ Owing to more abundant growth of stocks of the *UC* strain, this culture was used to study the influence of size of the amoebic inoculum. As Table II

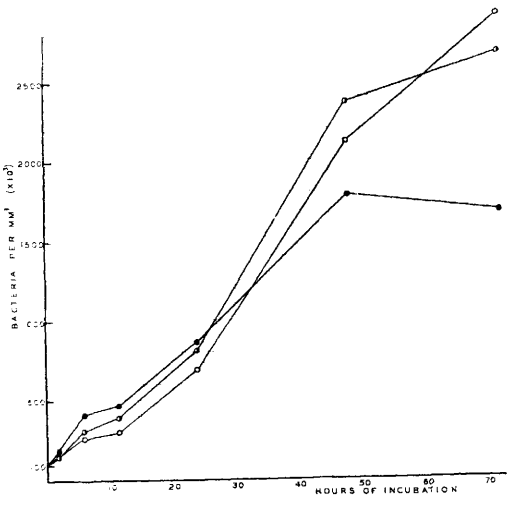

FIG. 2.
The effects of ethanol and prodigiosin on the growth of *Aerobacter aerogenes*.
● regular test medium.
Half solid right circle—0.75% ethanol added to test medium.
○ 0.75% ethanol + 1:100,000 prodigiosin added to test medium.

‡ This same relative reaction has been observed in this laboratory in the case of recognized amoebacidal agents. The implications for drug-testing procedures will be discussed elsewhere.

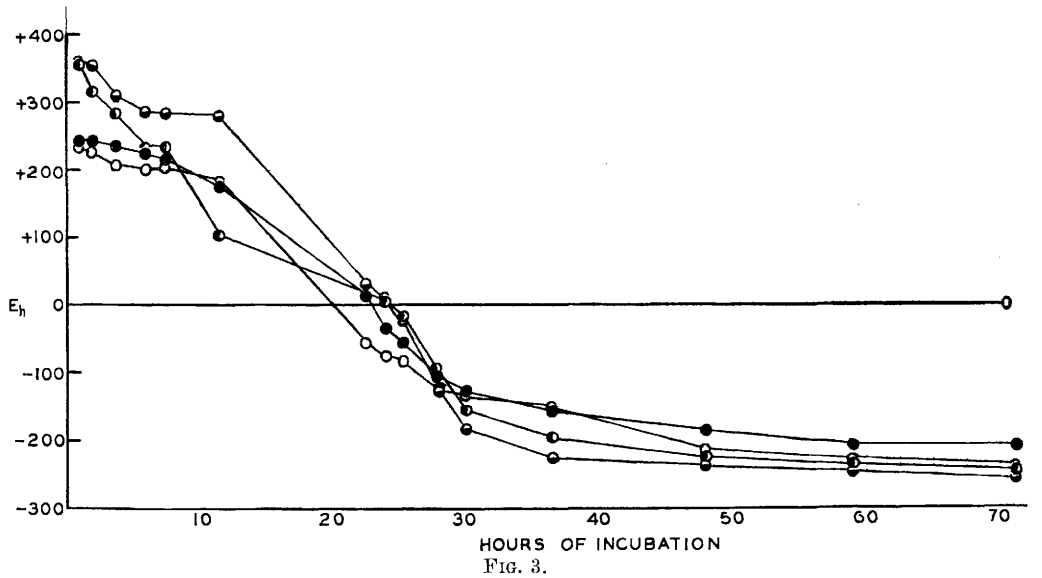

FIG. 3.

Changes in oxidation-reduction potential (E_h) of monobacterial cultures of *Entamoeba histolytica* (NRS strain with *Aerobacter aerogenes*), in different concentrations of prodigiosin.
● Control
Solid left half circle—1:1,000,000
Solid lower half circle—1:400,000
○ 1:200,000

shows, relatively large amoebic inocula resulted in greater resistance to prodigiosin at 1:200,000, and this could not be attributed to the bacteria. No survival was recorded for either combination at 1:100,000, regardless of size of inoculum, and we regard this as the lowest amoebacidal concentration of prodigiosin under our experimental conditions. Effects were clearly discernible against individual amoebae at 1:2,000,000; they became highly vacuolated and motionless, and acquired a pinkish tint. At higher concentrations the amoebae were lysed rapidly, so that even after 12 hours few carcasses persisted.

The effects on bacteria are seen most clearly in monobacterial cultures. There was no evidence of inhibition of *A. aerogenes* either by prodigiosin or ethanol in the concentrations employed (Fig. 2). Also in the case of the *UC* mixed flora, large populations of viable bacteria were present even at the highest concentrations of prodigiosin.§

The maintenance of low (*i.e.*, negative) oxidation-reduction potentials is a prime requisite for viability and growth of intestinal amoebae(17,18). Bradin and Hansen(19) report E_h values of approximately −200 mv for their control cultures. Since the redox potential of cultures is controlled chiefly by the bacterial flora(18), it would be expected from the present study that control and experimental cultures should agree quite closely in this regard. Fig. 3 and 4 disclose this to be true, and effectively rules out the possibility that amoebacidal action can be attributed to unfavorable potentials. It may be noted that the potentials fell more slowly in the monobacterial cultures, requiring some

§ Dr. E. Hansen has kindly tested a sample of our prodigiosin against three monobacterial cultures of *E. histolytica*, involving respectively organism *t*, *Clostridium perfringens*, and the streptobacillus of Shaffer and Frye. Dr. Hansen concluded (in litt.) that "Prodigiosin, in alcoholic solution, is amebacidal at 1:100,000, the alcohol present (0.5% ethanol) contributing to the toxic action," especially through marked inhibition of the bacterial associates. Our most complete bacterial analyses were conducted after receipt of this report. The flora in our cultures clearly did not respond in the same way as above, but rather in the manner reported by Hettche for members of the gram-negative intestinal flora.

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18. Chang, S., *Parasit.*, 1946, v37, 101.
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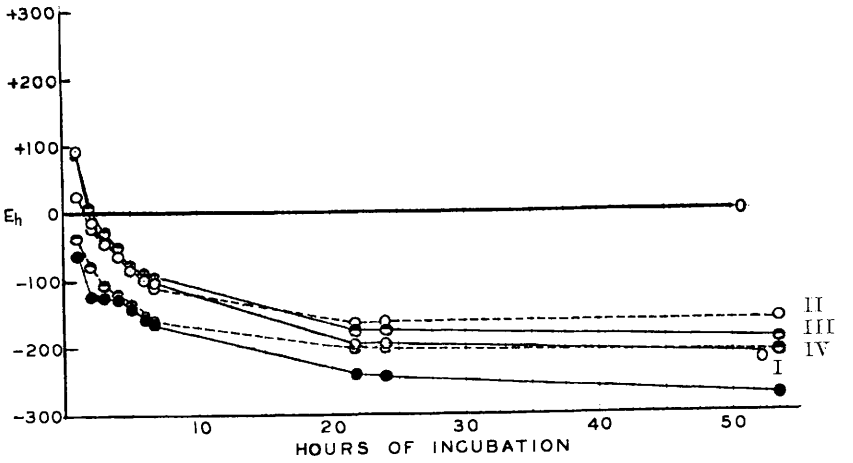

FIG. 4.

Changes in oxidation-reduction potential (E_h) of cultures of *Entamoeba histolytica* (*UC* strain with mixed flora) in different concentrations of prodigiosin, with different amoebic inocula.

● Control.
I—1:200,000 (102,000 amoebae)
II—1:200,000 (391,000 amoebae)
III—1:100,000 (102,000 amoebae)
IV—1:100,000 (391,000 amoebae)

30 hours to reach −200 mv in comparison to 10 hours for the *UC* strain, but in all cases controls and experimentals behaved similarly. The pH of all cultures was held in the range pH 6.8 to 7.2 through the use of well-buffered media.

Summary and conclusions. (1) Prodigiosin was employed in the range 1:20,000,000 to 1:100,000 against two distinct strains of *E. histolytica.*

(2) The monobacterial culture (*NRS* strain grown with *A. aerogenes*) proved more susceptible, failing to survive at 1:400,000. The mixed culture (*UC* strain) failed to survive at 1:100,000, even with relatively huge inocula of amoebae.

(3) Analysis disclosed that the bacterial flora, oxidation-reduction potentials and pH were not adversely affected by the experimental treatment.

(4) The general conclusion is reached that prodigiosin exerts a profound, direct action against *E. histolytica in vitro.* This is apparently the first record of proved direct action of an antibiotic against *E. histolytica.*

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Further Studies on the Thermostable Leukocytosis Factor of Exudates. (18204)

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The rise in the number of circulating leukocytes with inflammation is referable to at least two factors released by cells injured at the site of injury: (1) a thermolabile leukocytosis-promoting factor (the so-called LPF) and (2) a thermostable leukocytosis factor which is closely associated with the

* Aided in part by a grant from the National Advisory Cancer Council, U. S. Public Health Service. This is No. 42 of the series "Studies on Inflammation."

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