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]

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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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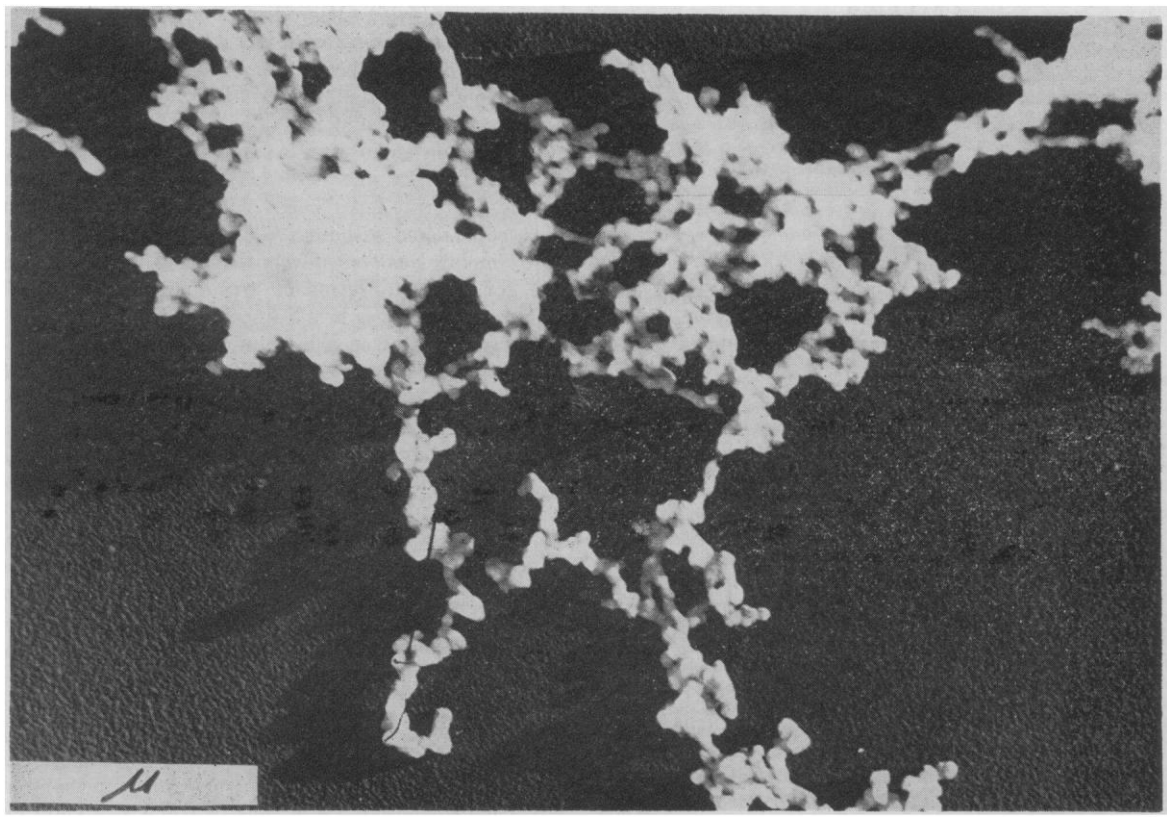

FIG. 1. Residual chicken chromosomes after one extraction with *M* NaCl, bulk-fixed with 2% aqueous chromic acid, shadowed with chromium at 10°.

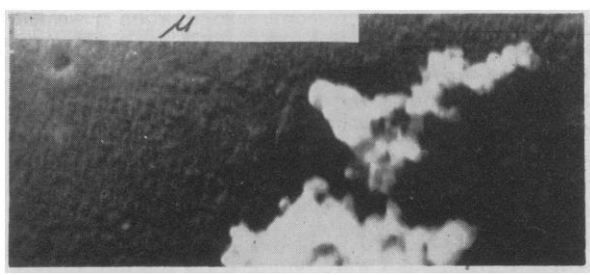

FIG. 2. Residual chicken chromosomes as in Fig. 1 but after 5 extractions with *M* NaCl.

extractions with *M* NaCl. This absorption was used as a measure of the removal of matrix (desoxyribonucleohistone) from the chromosomes. The data of 7 series of runs showed incomplete extraction of matrix in the first few washes, in which the volume-ratio of *M* NaCl to whole chromosomes was about 50; the transmission approached that of the *M* NaCl only after 5 or more extractions.

As a preliminary indication of the results obtained, Fig. 1 shows the mixture of fine twisted filaments and opaque areas characteristically seen in material after a single extraction; the supernatant was practically opaque at 2,600A. These filaments range from about 200 to 600A in diameter; the appearance of the interspersed opaque areas, usually present, suggested that they represented an amorphous material and were not due simply to poor dispersion of meshes of filaments. A second extraction of this material gave a supernatant with transmission only about half that of the vehicle alone. After 5 extractions of similar material, the supernatant displayed the same transmission as *M* NaCl, and micrographs showed apparently clean strands with none of the opaque areas cited. A seemingly coiled end of such a strand is shown in Fig. 2. Similar contorted clean strands obtained after 12 extractions in another run are shown in Fig. 3; the corresponding supernatant displayed a transmission near that of the vehicle. These bodies show continuous filamentous nature and a wide range of diameters (about 200–1,000A). In Fig. 4 is shown a strand from the material extracted 5 times that disclosed unique structural features; intertwining of 2 strands about 400A in diameter to form one about 900A in diameter is seen, and good prints show indications of transverse banding on the 400A filaments, with a period of about 100A. Although this crossbanding was not regularly observed in these preliminary preparations, it seems likely to be significant as it is not apparently to be confused with the structure of the supporting film.

Mock runs following these preparative techniques in all respects, but with omission of the cells, disclosed no structures to be confused with chromosomal material either in the optical or electron microscopes for stages including mock whole chromosomes, and mock

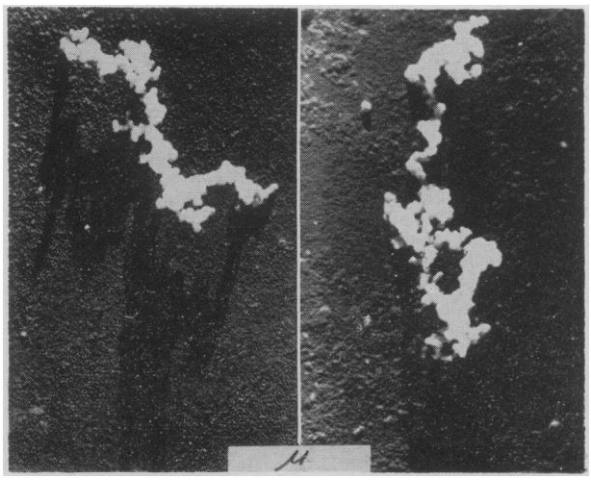

FIG. 3. Residual chicken chromosomes as in Fig. 1 but after 12 extractions with *M* NaCl.

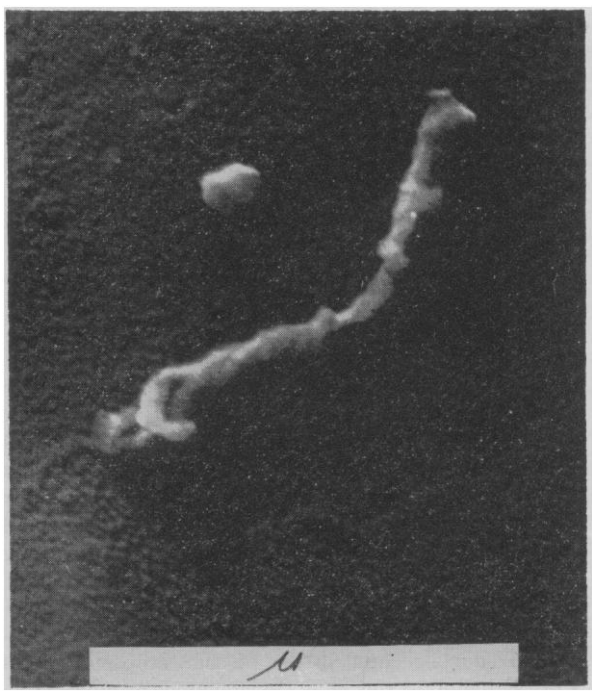

FIG. 4. Residual chicken chromosome as in Fig. 1 but after 5 extractions with *M* NaCl.

first and second residual chromosomes. Later, similar runs offering a partial control employed anuclear sheep red cells and likewise disclosed in the phase microscope no objects similar to chromosomes. Contaminations introducing some absorption at 2,600A were encountered in some mock runs, but these were not of a magnitude to confuse the qualitative picture given here. Despite precautions, an unidentified rodlike body, typically 500A in diameter and from ¼ to 1 μ in length, occasionally double, was seen both in controls and in some of the preparations. The known remarkable appearance of carbon (*4*), and the appearance of casual laboratory dirt in apparently continuous "coiled" filaments, with uniform diameters of the order of 200A were verified in other controls. Such material when introduced here was of lower density than the chromosomal material when viewed on the fluorescent screen of the microscope—a fact that was a qualitative aid to the chromosomal identity of the material under study.

These preliminary results suggest the interest of this technique for the study of the fine structure of chromosomes. The uncertainty of the size range of chicken chromosomes, many of which lie at the limit of resolution of the optical microscope, precludes at present the logical synthesis of such fragmentary information into a picture of the whole chromosome. There is also some further current uncertainty of the identity of such isolates, although we see here obviously nuclear material that seems to satisfy many accessible criteria (form, staining) of chromosomal identity. Thus, in a recent preliminary report, Lamb (*20*) has concluded from optical, phase, and electron microscopic studies of calf thymus cells disrupted for various intervals by sand grinding, by Waring Blendor, or by treatment in the annulus between 2 rotating glass cylinders that the isolated whole "chromosomes" so obtained are but fragments of drawn-out nuclei, found to be single rather than double in the electron microscope. Further, Calvet *et al.* (*15*) have described in a short note meshes of fine fibers obtained by vacuum rupture of such cells, rather than discrete whole chromosomes, and have interpreted these as the classical chromatin net. These workers also interpreted particles obtained through use of the Waring Blendor as random fragments of this network rather than as preformed structural units. Such uncertainties are sufficiently important in the eyes of some workers (*21*) to render uncertain the localization of a protein fraction recently obtained from whole "chromosomes." These views are difficult to reconcile with the careful original work of Mirsky and Ris (*14*), and with the filamentous structures here obtained by extraction of such isolated whole chromosomes, an example of which shows clearly double structure after removal of obscuring matrix (Fig. 4). Most recently, Brues and Rietz (*22*) have presented evidence from the examination of nuclei partially disintegrated by a colloid mill that the chromosomelike structures they isolated are indeed preformed structures in the nucleus. Perhaps one might take heart from the aphorism (*23*) "The nucleus is the chromosomes"! But it is manifest that much further work is needed.

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Ethanol from Methanol[1]

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A method for converting an alcohol to the primary alcohol containing one carbon atom more than the original (homologation) under conditions resembling those employed in the oxo reaction has recently been reported (*1*). The conversion is achieved by treating the alcohol with synthesis gas (carbon monoxide and hydrogen) at 180°–185° in the presence of a cobalt catalyst. The order of reactivity of various starting alcohols was found to be tertiary > secondary > primary. It has now been found that the simplest primary alcohol, methyl alcohol, is anomalous in that it reacts with synthesis gas more rapidly than secondary alcohols. Ethyl alcohol is the chief product of the reaction, and the conversion presents an interesting new route to this alcohol that may have important theoretical and commercial implications. The interest in the reaction from a mechanism point of view arises from the fact that it is impossible for methanol to react via an olefin intermediate.

In one experiment, 2 moles (64.1 g) of methyl alcohol and 4 g of dicobalt octacarbonyl, $[Co(CO)_4]_2$, were placed in a stainless steel autoclave, which had a maximum free space, when empty, of 468 ml. Synthesis gas ($1H_2:1CO$) was added until the pressure reached 3,500 psi, and the autoclave was then heated with rocking to 185° within 65 min. The maximum pressure obtained was 5,100 psi at 181°. The temperature of the autoclave was held at 183°–185° for 4 hr, during which time the pressure dropped to 2,410 psi. The reaction vessel was cooled to room temperature and then repressured to 3,000 psi with 1:1 synthesis gas. The temperature was raised to 185° once more (maximum pressure, 4,560 psi at 167°) and held at this temperature for 4 hr, during which time the pressure dropped to 3,150 psi. The total pressure drop corresponded to approximately 3.6 moles of gas, or 2.3 moles of gas per mole of methyl alcohol converted. After cooling to room temperature overnight, gas samples were taken (over water), and the residual gases vented to the atmosphere. Mass spectrometric analysis of the carefully fractionated mixture showed that 49.0 g (76.4%) of the methyl alcohol had reacted to give the products shown in Table 1. Yields are based on converted methanol.

TABLE 1

PRODUCTS FROM THE REACTION OF METHANOL WITH SYNTHESIS GAS

Compound	Yield (%)
Methyl formate	2.0
Methyl acetate	9.0
Ethyl alcohol	38.8
Ethyl acetate	6.3
Propyl alcohol	4.7
Butyl alcohol	0.9
Methane	8.5
Propyl acetate	0.1
Total	70.3
Water	90.8

Approximately 2.0 g of the distilled product could not be identified. A semisolid residue (6.4 g) containing metallic cobalt and organic material was left in the distillation flask. Acetaldehyde was identified in the lower-boiling fractions as its 2,4-dinitrophenylhydrazone, mp 148.3° – 149.2°, but no estimate of its total concentration could be made.

The absence of appreciable quantities of higher alcohols in the products from the reaction of methanol indicated that ethanol probably would react sluggishly in the homologation reaction. This was confirmed by experiment; a 4.1% yield of *n*-propanol and small quantities of butyl alcohols were secured after a long period of reaction. The variety and distribution of products obtained from methyl alcohol in the homologation reaction have certain similarities to the oxygenated products secured in the Fischer-Tropsch process (*2*). Ethanol is the chief oxygenated product from the Fischer-Tropsch reaction, and relatively small quantities of methyl, propyl, and butyl alcohols are found. The preponderance of ethanol and the relatively small amounts of methanol, *n*-propanol, and butanols in the Fischer-Tropsch process products may be significant in comparison with the rapid conversion of methanol and the slow reaction of ethanol characteristic of the homologation reaction.

The yield of ethanol from methanol can probably be increased by increasing the hydrogen to carbon monoxide ratio of the synthesis gas. It is not necessary to use preformed dicobalt octacarbonyl in this reaction; any salt of cobalt yielding the carbonyl under the conditions of the reaction will serve as an effective catalyst (*1*). A small continuous unit has been con-

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