4. Niobrara formation (Cretaceous) chalky shale, erosional top; 32 ft exposed above terrace level along east side Indian Creek.

The Nebraska Geological Survey, the University of Nebraska State Museum, and the Department of Geology at the University of Nebraska have approved the foregoing restriction of the term Grand Island and the adoption of the new name Red Cloud sand and gravel, and these names will be used in future Nebraska publications as herein defined. It may be necessary to apply the hyphenated term Red Cloud–Grand Island to some subsurface intervals in the Pleistocene basin areas where the two formations cannot be separated satisfactorily. However, the subsurface studies of the Nebraska Geological Survey indicate that the Red Cloud–Grand Island interval in many test holes can be separated into a lower sand and gravel grading from coarse-textured at the base to fine-textured above, overlain by a higher sand and gravel with similar textural characteristics.

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Apparatus for the Culture of Bacteria in Cellophane Tubes

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Highly potent botulinum toxin has been produced by Wentzel and Sterne, who grew *Cl. botulinum* in cellophane bags immersed in corn-steep liquor (*1*).

In a previous publication (*2*), we have shown the possibility of obtaining high-titer tetanus toxin with the above-mentioned technique but using a different medium. During the course of this investigation, we were led to increase the ratio: cellophane surface/vol of medium; the result of our attempts is evident in the apparatus shown in Fig. 1, which can be described as follows:

A 4-ft length of 64-mm diam Pyrex tubing is fitted at both ends with flared openings for No. 9 rubber stoppers; two side outlets for ¼-in. rubber tubing

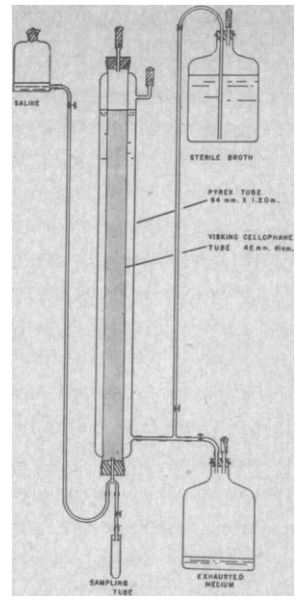

FIG. 1.

are also provided at the two ends of the glass tube.

Into this glass tube is fitted a 42-mm diameter Visking cellophane tubing with the ends intussuscepted over the flared openings of the glass tube, which is now fitted with one-holed rubber stoppers. One of these (which is called the bottom) receives a Y-tube for supplying the inner tube with saline. The other stopper (top) is fitted with an air filter (funnel type).

Rubber tubing connects one end of the Y-tube to a 2-liter bottle filled with saline; the other outlet of the Y-tube is fitted with a device for aseptic sampling.

Similar rubber tubing is used to connect one branch of a T-tube on the lower side of the outside (glass) tube to a 12-liter bottle containing the fresh culture medium. The other outlet of the T-tube is also equipped with a 12-liter bottle for collecting the exhausted medium.

The upper side outlet of the outside glass tube receives an air filter. All rubber-to-glass connections are wired.

The whole apparatus is sterilized in a 6-ft long autoclave for 1 hr at 120° C.

After sterilization the apparatus is hung from the ceiling in an incubator room. Seed culture is introduced into the cellophane tube through the lower Y-tube bringing the saline. The culture medium is then introduced outside the cellophane by means of the lower side outlet.

Bacterial growth is initiated in the saline-dialysate mixture in 24–48 hr, whereas the outside medium remains clear and free from those metabolic products which cannot dialyze—e.g., tetanus toxin. Should the otherwise clear medium become cloudy, one suspects either contamination or a defect in the cellophane. (These can, however, be detected in advance by filling with water before setting up.)

Periodic replacement with fresh broth of the more

or less exhausted medium outside the cellophane tube supplies the bacterial culture with a constantly renewed supply of nutritive substances as well as removes some of the dialyzable metabolic products which might restrain growth. Thus much heavier growths are obtained than in the usual bottle or flask cultures.

It was soon realized that this apparatus has several other advantages:

1. From the practical point of view, the possibility of renewing the exhausted medium constantly as well as removing periodically part of the bacterial culture, induces us to envisage the production of bacterial cells and bacterial products (toxins, enzymes, etc.) by a continuous process.

2. From the theoretical point of view, the possibility of sampling periodically the contents of both the inner and outer tubes permits us to follow chemically and immunologically the processes concerned with this particular bacterial metabolism.

In a subsequent note, we hope to establish that the heavy growths obtained in this new method result from an extended multiplication phase of the bacterial culture.

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An Improved Iodine-staining Technique for Routine Laboratory Diagnosis of Intestinal Protozoa[1]

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An exceedingly simple and rapid iodine-staining technique has been devised by which trophozoites as well as cysts of intestinal protozoa may be stained. For routine identification, the method appears to be an improvement over currently used techniques, including hematoxylin procedures.

An "MIF" solution, so designated because of the presence of merthiolate, iodine, and formaldehyde, is made up as follows:

.10 ml Lugol's iodine solution (Merck Index)	10 parts
.15 " Formaldehyde (USP)	15 "
.75 " Tincture merthiolate 1: 1000 (Lilly)	75 "

The mixture is placed in a standard Kahn tube and is ready for use immediately. The 1-ml amount is sufficient to fix and stain 20–25 stool preparations. For consistently good results the tube should be stoppered when not in use, and the solution should be discarded after standing for 6–8 hr.

The stain is used as follows: On one end of a glass microslide the usual unstained smear is made by mixing a small amount of freshly passed feces in a drop of normal saline. On the other end of the same slide is placed a drop of distilled water (half the size of the saline drop used for the unstained smear), and to this a drop of equal size of MIF fixing and staining solution is added. About twice as much feces as that used in the simple saline smear should be very thoroughly mixed in the second preparation. Cover slips are placed over both preparations. The total size of either drop should just suffice to fill the under surface of the cover slip, for films that are too thick are unnecessarily difficult to study. The usual microscopic examination of the saline smear is then made, followed by examination of the stained-fixed preparation for nuclear and cytoplasmic detail such as one seeks in iron-hematoxylin mounts. A blue light filter is useful to enhance differentiation of cytoplasmic and nuclear detail.

Fixation of the various species of amebic trophozoites occurs without observable damage and without the loss of organisms that so frequently happens in iron-hematoxylin preparations. Often pseudopodia are seen fixed in extended position. Staining of nuclear and cytoplasmic details in trophozoites of freshly passed stool specimens is of good diagnostic quality. The cytoplasm immediately becomes differentiated, usually first taking a yellowish coloration, which then changes to salmon pink. Nuclear elements stand out in contrast, taking a brownish to almost jet-black stain. Often granules of the nuclear ring are individually defined. Organisms from cases of amebic dysentery stain particularly well, showing ingested red blood cells more readily identifiable than in fresh living unstained preparations.

The above results have held for trophozoites of all human species of amebae, including limited observations on *Dientamoeba fragilis*. With trophozoites of flagellates, cellular morphology and nuclear elements are well demonstrated. Individual flagellae are usually readily identifiable.

The color sequence of the cytoplasmic staining reaction (unstained–yellow–pink) of cysts of amebae or flagellates is slower and more variable in final coloration than is the case with trophozoites. The cyst cytoplasm (especially *Endamoeba coli*) may even remain unstained, but generally assumes an iodine-yellow stain and finally a salmon pink as seen with trophozoite forms. However, regardless of variability of cytoplasmic staining reaction in cysts, the important diagnostic nuclear elements, chromatoidal bars, glycogen vacuoles, etc., are of a definition which permits immediate specific identification.

Variations in trophozoite- and cyst-staining occur between individual organisms as well as between species. This is apparently due to inherent differences in permeability. The formula is designed, however, to cover the usual range of permeability differences. Should a greater nicety of differentiation be desired, the iodine may be increased to 12.5 or 15 parts with a proportionate decrease in merthiolate content. Although this is not frequently required for routine

[1] A preliminary report.