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.

diagnostic purposes, initial observations should employ trials at the three iodine strengths to demonstrate the full potentialities of the procedure.

Certain precautions must be observed. Fresh Lugol's solution should be prepared weekly, and the MIF formula should be discarded after 6–8 hr usage. To date general staining failures have invariably been due to failure to observe these two precautions. It should also be emphasized that individual degenerate organisms occur in all stools, and in increasing numbers when stools are not examined promptly after passage. Such organisms will not take an acceptable stain with this technique or with any other staining procedure. Freshness of the stool specimen is therefore all-important for good results.

Influence of Testosterone on Nucleic Acid Phosphorus of Rat Seminal Vesicle

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Few data exist in the literature on the nucleic acid content of hormone-controlled organs (*1, 2*). None are found on androgen effects on male accessory reproductive organs. In this paper we wish to report the effect of testosterone propionate on the nucleic acid phosphorus content of rat seminal vesicles.

Rats with a mean weight of 220 g (range 150–280 g) were used. The animals were divided into three groups. Group I consisted of control rats, group II of rats castrated 8 days previously, and group III of castrated rats which, after 8 days, were injected with four 5-mg doses of testosterone propionate in oil on alternate days, and sacrificed on the ninth following day.

Histological study demonstrated that castration produced an intense cell atrophy, reversed by testosterone administration, as described by Moore and coworkers (*3*).

Animals were killed by a blow on the head, both seminal vesicles weighed on a torsion balance, and homogenized with distilled water in a glass homogenizer. Ribonucleic acid phosphorus (RNAP) and desoxiribonucleic acid phosphorus (DNAP) were determined in 0.8-ml aliquots of 2% homogenates by Schmidt and Tannhäuser's method (*4*), as modified by Davidson *et al.* (*5*). Phosphorus determinations were performed, according to Pereira (*6*), with some modifications. Reproducibility of the method used was tested, and variation coefficients around 3.5% for DNAP and 5.0% for RNAP were obtained.

Significance of differences between the means of the three groups was studied by the F test (*7*), and that of pairs of means by the t test (*8*). Values of F and t corresponding to probabilities of 0.01 or less were considered as indicating statistically significant differences between the means.

Data obtained are presented in Table 1. F tests on data of each of columns 2, 3, and 4 showed that they are samples of different populations; t tests for pairs of means of each of columns 2, 3, and 4 indicated statistically significant differences, except for the DNAP means for groups I and III ($P > 0.2$). These results cannot be due to modifications in the water content of the seminal vesicles, as the ratio dry wt/wet wt was found to be similar for the three groups.

The greater DNAP content of seminal vesicles of group II (castrated) animals as compared with those of the two other groups is probably due to cell atrophy, with increase in cell and nuclear population per unit weight. The decrease of RNAP and RNAP/DNAP ratio in seminal vesicles of group II (castrated) rats, and their higher values in group III (castrated and injected) rats, as compared with group I (control) rats, are in agreement with the views of Brachet (*9*) and Caspersson (*10*) on the relation between ribopolynucleotides and protein synthesis. It is concluded that testosterone propionate can influence nucleic acid phosphorus content of rat seminal vesicles.

TABLE 1

NUCLEIC ACID PHOSPHORUS CONTENT OF CONTROL, CASTRATED, AND CASTRATED TESTOSTERONE-INJECTED RAT SEMINAL VESICLES

Group	Col 1	Col 2	Col 3	Col 4
	Mean wt of seminal vesicles (range)	Mean DNAP* content (range)	Mean RNAP* content (range)	Mean RNAP/DNAP ratio (range)
I Control (7 rats)	220 (130–329)	25.4 ± 2.45† (16.7–31.4)	48.5 ± 14.15† (29.0–71.7)	1.94 ± 0.51† (1.07–2.45)
II Castrated (8 rats)	96 (48–209)	46.9 ± 8.22 (35.1–61.0)	25.4 ± 2.90 (21.6–30.2)	0.55 ± 0.08 (0.44–0.65)
III Castrated and injected	380 (292–461)	29.3 ± 1.78 (21.1–36.1)	77.2 ± 9.45 (65.2–87.6)	2.67 ± 0.25 (2.34–3.09)

* Micrograms/100 mg fresh weight.
† Standard deviation.

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