0.1 *M* citrate buffer at pH 4.5. After being rinsed in distilled water, the stained sections were dehydrated in ethyl alcohol and mounted in xylene-damar .

Normal solutions of HCl, $HClO_4$, and CCl_3COOH were prepared from C.P. reagents by electrometric titration with *N* NaOH. The pH of the normal acids at room temperature was 0.05, 0.00, and 0.29, respectively. Mounted tissue sections were incubated in each of the acids and in distilled water under various conditions of time and temperature prior to staining. The basophilia of each of the tissue components under study was then compared with that exhibited in stained sections that had not been incubated in either acid or distilled water. It should be noted that the depth of staining of each component in untreated sections was recorded as "three plus" (+++), although in reality the basophilia of the different substances varied in intensity. The experimental conditions and observations are summarized in Tables 1 and 2.

It can be readily seen from Table 1 that the method of fixation considerably alters the extractability of RNA by cold $HClO_4$. This observation explains the failure of the method when applied to paraffin sections of Zenker-fixed tissue (*8*) after its initial successful use with alcohol-fixed material (*6*). The increased acid-resistance of RNA in paraffinized fixed tissues, however, is due in large part to the embedding process, since RNA is readily removed from frozen sections of Zenker-fixed material (*8*). The present data concerning the effects of time and temperature on the action of $HClO_4$ on the stainability of nucleic acids (Table 2) are in substantial agreement with the observations of previous investigators in that (1) the extractability of RNA varies in different tissues, (2) the rate of extraction increases with temperature, and (3) at elevated temperatures both RNA and DNA may be completely removed from the sections. In addition, the present studies demonstrate that the stainability of acid mucopolysaccharides is similarly affected. The ready extractability of epithelial mucus, in particular, precludes the use of this method on tissues which may contain both cytoplasmic RNA and mucin; e.g., vaginal epithelium, anterior lobe of the pituitary, salivary glands, etc. At present, then, it would be advisable to use the ribonuclease technique under such circumstances.

The results obtained with HCl and CCl_3COOH are, in general, qualitatively similar to those obtained with $HClO_4$, although under the same conditions of time and temperature the amounts of basophilic material extracted by the three acids differed. It is of interest to note that DNA, heparin, and chondroitin sulfate apparently are hydrolyzed at a slower rate in HCl than in either $HClO_4$ or CCl_3COOH (most evident at 37° C), whereas HCl is almost equally as effective as $HClO_4$ in removing RNA. This property of HCl should be advantageous in the differentiation of RNA from DNA, but the ready solubility of epithelial mucus may limit the wider application of the method as noted above.

The present experiments have provided specific data on the acid-extractability of but a few representative acid mucopolysaccharides and nucleic acids in PAF-fixed mammalian tissues. The results, however, indicate that HCl, $HClO_4$, and CCl_3COOH are nonspecific in action and may remove members of both these groups of basophilic compounds, depending on the circumstances. In the histochemical differentiation of RNA from DNA, acid extraction may be substituted for ribonuclease digestion only in the absence of extractable acid mucopolysaccharides and only when the conditions of time and temperature are empirically determined for the particular organ to be studied and fixative used.

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The Accuracy and Convenience of Silicone-treated Microliter Pipettes

Edward L. Duggan and Kendric C. Smith

Department of Biochemistry, University of California School of Medicine, Berkeley

The present deserved popularity of paper chromatographic techniques has resulted in the widespread use of microliter pipettes, with occasional misunderstanding of their proper usage or inherent accuracy. Existing methods of calibration (*1*) of pipettes of less than 100 µl capacity are based upon the weight of mercury contained in the dry pipette. Thus, such pipettes are capable of accurate and reproducible content but not of delivery.

The advent of silicone mixtures (*2, 3*) that may be applied to general glassware to produce water-repellent surfaces promises great improvement in quantitative techniques, along the lines indicated by Gilbert (*3*). This paper enumerates several advantages which accrue to microliter chemistry by the use of water-repellent coatings.

To test the efficacy of such coatings in permitting the use of 10-µl pipettes for delivery, two such pipettes (Microchemical Specialties No. 280B) were treated with Desicote according to the procedure outlined in the manufacturer's bulletin (*2*).

A calibration of the pipettes under conditions of use was accomplished using potentiometric titration of the acid delivery from each pipette. A calibrated 10-ml microburette was used for the titrations, the capillary tip dipping into the solution being titrated. The sulfuric acid delivered by the micropipettes was 13.54 *N*; titrant alkali was 0.018 *N*. The titration ratio between acid and alkali was determined for 1.00-ml samples of the diluted acid after accurate 1 : 100 dilution, using Normax pipette and volumetric flask. Replicate titers to pH 5.3[1] were 7.361, 7.358, and 7.360 ml, yielding an average of 7.360 ml ± 0.003 SE. Considering the dilution factor, the average titer thus represents a 10.00 µl delivery of the 13.54 *N* acid. The micropipette volume may be calculated from the relation: Average volume = (average titer in ml/7.360 ml) × 10.00 µl. The chief source of error in this calibration method is the error of dilution of the concentrated acid.

TABLE 1
COMPARATIVE CALIBRATION OF 10-MICROLITER PIPETTES

Pipette No.	Mercury content		Acid delivery	
	Replicates	Av ± SE*	Replicates	Av ± SE*
1	3	9.968 ± .006 µl	2	9.961 ± .009 µl
2	4	9.858 ± .020	2	9.841 ± .000

* Standard error = $\sqrt{\frac{\Sigma(d^2)}{N(N-1)}}$

For comparison, the pipettes were also calibrated with mercury by the method of Kirk (*1*). The results given in Table 1 show that the two methods agree to better than 0.1% and demonstrate an equality between content and delivery for silicone-treated micropipettes.

Complete delivery of the concentrated acid from the treated pipettes is indicated by the close agreement among replicate titers of single deliveries (without rinses), and by the agreement obtained between mercury content and acid delivery. In order to confirm complete delivery, the second pipette was tested for "holdup" of acid by rinsing with several portions of the titration mixture after the end point of pH 5.3 was reached. This caused a shift of less than 0.03 pH unit below the end point value. A retention of 10^{-5} µl of 13.54 *N* acid on the inner surface of the pipette would result in lowering the pH of the unbuffered titration mixture by more than 0.3 pH unit. Therefore, the "holdup" of acid by this pipette was less than 10^{-6} µl.

The acid delivery method of calibration may perhaps be preferred by workers who have used neither method previously. It has the advantage of simulating conditions of pipette use by the operator, thus providing a check of the operator's technique at the time of calibration. Presumably the potentiometric titration of acid delivery could be replaced by titration using the double indicator of Hawes and Skavinski (*4*) without great loss of accuracy for the calibration procedure.

Several precautions should be noted with regard to the use of a silicone on pipettes. The coating becomes imperfectly water-repellent unless carefully stored completely dry or completely wet so that occasional repetition of the silicone treatment is necessary. Unless it is subsequently demonstrated that the technique of silicone removal and recoating does not lead to changed pipette volumes, calibration after each cycle is necessary.

Silicone coating of microliter pipettes of the self-adjusting type (Microchemical Specialties No. 282A–283B) provides even greater convenience in their use. Certain of these pipettes, before treating with silicone, will hold several microliters of drainage liquid between the upper capillary and the bulb, which is difficult to recover by rinsing. After siliconing, such pipettes drain completely so that no visible trapping of the drainage volume occurs.

It is apparent from these results that water-repellent coating, such as that provided by Desicote, may introduce an era of accurate and convenient volume measurement for microliter chemistry as well as for macro-analysis. Such a coating provides equality between content and delivery for these 10-µl pipettes, and presumably for larger micropipettes as well (*3*).

[1] The end point was arbitrarily taken as pH 5.3 to minimize carbon dioxide absorption during the titration.

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Propagation of a Strain of *Endamoeba histolytica* in Tissue-bearing Culture

James G. Shaffer[1] and Henry S. Sienkiewicz[2]

Department of Microbiology, School of Medicine, University of Louisville, Louisville, Kentucky

It is not known whether *Endamoeba histolytica* can invade host tissues by virtue of its own invasive properties or whether in some way certain bacteria lend assistance in the invasion of the tissues and, perhaps, contribute to the production of the characteristic lesions seen in patients with amebiasis. The experiments to be reported here were undertaken with the thought that if *E. histolytica* could be established in cultures containing animal tissues of various kinds, free of bacteria, it would be possible to study (*a*) the mode of entry of the amebae into the tissues, (*b*) the

[1] Present address: The Chicago Medical School, 710 S. Wolcott Ave., Chicago 12, Ill.

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