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Editor, Lee G. Luna, D. Lit., H.T. (ASCP)

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pH Simplified

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Editor's Note: This is the last of a three part article. Part one appeared in *Histo-Logic*, Vol. IX, No. 2, April 1979; part two appeared in Vol. IX, No. 3, July 1979.

The pH Meter:

A pH meter is a specialized voltmeter which has two fundamental requirements. First, it must be able to function accurately when measuring the voltage of extremely high resistance electrodes. Second, one must be able to change its sensitivity as a voltmeter to correspond to the pH/voltage characteristics of the electrode system.

Most modern pH meters use all solid state electronics with very high input resistance or impedance characteristics. These meters measure the voltage of the pH electrode system while drawing extremely little current.

Fortunately, the voltage change of a pH electrode varies linearly with pH units. At room temperature, a change of 1 pH unit causes a voltage change of about 60 millivolts (mV) or 0.060 volts. At 0°C (temperature at which water freezes) 1 pH unit change causes a 54 mV change. At 100°C, a 1 pH unit change causes a 70 mV change. Thus, a properly designed pH meter will have a temperature dial which varies the sensitivity of the meter to match the voltage from the electrodes.

Occasionally, specialized sensing electrodes fall short of delivering the full voltage which theory would predict. Accordingly, very versatile pH meters will also have an additional sensitivity control, called a slope control. This control, like the temperature dial, allows the analyst to vary the sensitivity of the meter to match the voltage from the electrodes.

The pH Standard:

The voltage from the pH electrodes at any given pH value can be predicted approximately. However, for highest accuracy, the pH electrode system can be dipped into a solution of known pH and then the meter adjusted to correspond to this pH value. This adjustment is called standardizing the pH system. The solution used is called a pH standard buffer solution.

Table 4

pH Value 25° C	Composition
1.68	Potassium Tetroxalate (0.05M)
3.56	Potassium Hydrogen Tartrate (Saturated)
4.01	Potassium Hydrogen Phthalate (0.05M)
6.86	Potassium Dihydrogen Phosphate (0.025M)
9.18	Borax (0.01M)
12.45	Calcium Hydroxide (Saturated)

The chemical composition of pH standard buffer solutions has been defined by the U.S. National Bureau of Standards. Such solutions may be prepared by a competent chemist or technician. They are also available from most pH meter manufacturers. Table 4 lists the more popular pH standard solutions.

For best accuracy, a pH meter should be standardized using a standard solution whose value is near that of the test solution. However, standardizing with the pH = 6.86 standard constitutes a good compromise when the test solutions cover a broad range of pH values. A pH meter may be standardized as follows:

1. Rinse electrodes with distilled (or deionized) water and blot dry.
2. Place electrodes in pH standard buffer solution.
3. Adjust pH meter temperature dial to the temperature of the standard solution.
4. Turn pH meter to "operate."
5. Adjust pH meter to the pH value of the standard, using the "standardize" control. **The pH meter is now standardized.**
6. Turn pH meter to "standby."
7. Remove electrodes from standard and rinse with water.

The pH Measurement Procedure:

Once the pH meter is standardized, the measurement procedure is as simple as this:

1. Rinse electrodes with distilled (or deionized) water and blot dry.
2. Place electrodes in test solution.
3. Adjust pH meter temperature dial to the temperature of the test solution.
4. Turn pH meter to "operate."
5. Read the pH of the test solution on the pH meter directly.
6. Turn pH meter to "standby."
7. Remove electrodes from test solution and rinse with water.

Redox Measurements:

The measurement of the oxidation-reduction potential of a solution is commonly called a redox measurement. This measurement gives an indication of oxidizing or reducing power of a solution. Since a pH meter is also a very good voltmeter, it can be used in making redox measurements.

The sensing electrode used in this measurement is usually platinum, although gold and silver have been used for special purposes. The reference electrode is the same as that used in pH measurements. The electrode potential is usually expressed in millivolts (mV). Thus, most pH meters have a (mV) scale, as well as a pH scale. Also, since the temperature coefficient varies with the particular redox couple being measured, the temperature control is deactivated during the (mV) measurement.

pIon Measurements:

In recent years electrodes similar to the pH electrode but specific for other ions have been developed. These include electrodes for ammonia, chloride, cyanide, nitrate and sulfide, to name a few. These electrodes may be used in combination with a reference electrode with any modern pH meter. The meter should be standardized in a solution of

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known pI of the ion of interest, just as in the pH standardization. The pI of a test solution can then be read directly on the meter as in the pH measurement.

Effects of Dehydrants and Clearing Agents on Improperly Fixed Tissue

An Editorial

Inadequate fixation of surgical specimens and subsequent exposure to dehydrants and clearing agents may cause irreversible changes in tissue. Alterations (nuclear shrinkage) may occur in the central areas, and the quality of the section rapidly improves toward the properly fixed margin or periphery.

Table 1, adapted from Lillie,¹ demonstrates time and temperature requirements for adequate fixation in formalin with specimens 4 mm thick. Lillie also states, "... an 8-9% formaldehyde solution (20% formalin) hardens tissue in 3 hours at 55° C." Conversely, in the typical surgical laboratory-type facility, most of the surgical specimens are exposed to the fixative for 6 to 8 hours. It is obvious from the foregoing that a great many surgical specimens are not adequately fixed prior to processing.

Table 1
Time and Temperature Requirements

Temperature	Specimen Thickness	Time
20-25° C	4 mm	48 hours
35° C	4 mm	24 hours

The following mini-report is the result of a study I conducted to determine the effects of dehydrating and clearing agents on tissue that was not adequately fixed.

Specimens of human skin were exposed to 6 dehydrants and 6 clearing agents for 4 hours. In order to secure an exaggerated representation of the effects, the specimens were fixed after exposure to the respective dehydrating and clearing agents. One specimen was fixed in phosphate-buffered 10% neutral formalin to serve as a control for comparison.

Figure 1 is the specimen properly fixed in phosphate-buffered 10% neutral formalin. The effects of dioxane are demonstrated in Figure 2. Chloroform and xylene, popular clearing agents, produced the marked alterations depicted in Figures 3 and 4, respectively. Dehydrants and clearing agents will produce significant alterations in tissue specimens that are inadequately fixed.

The problems of inadequate fixation and subsequently altered tissue structures mentioned can be reduced by the

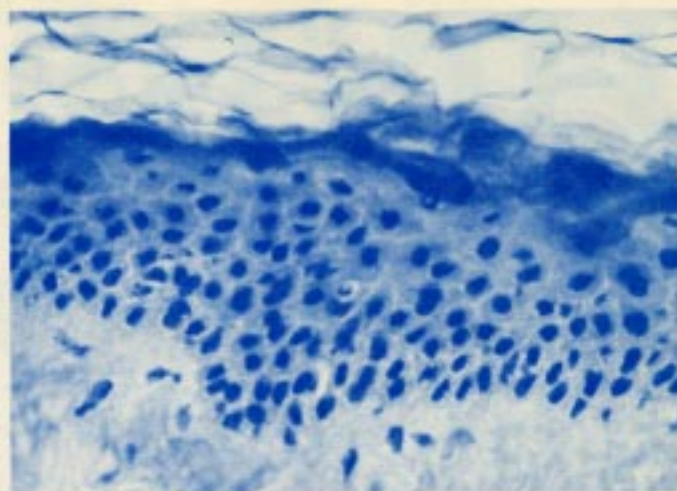


FIGURE 2:
Effects of dioxane: Dense nuclear structures (H&E x575).

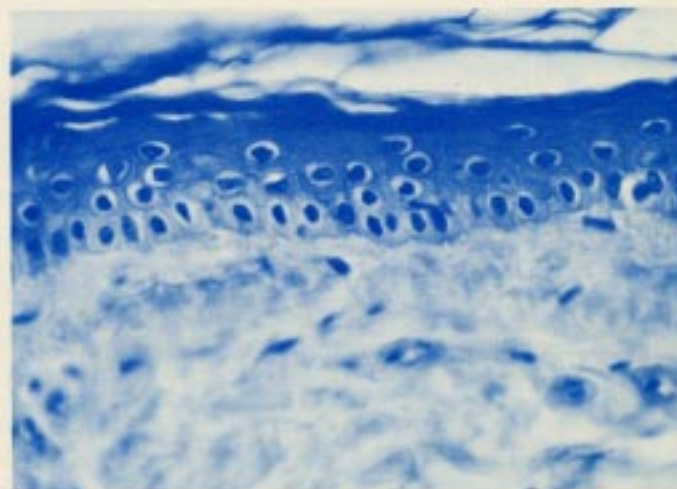


FIGURE 3:
Effects of chloroform: Marked nuclear shrinkage, no detail of nuclear chromatin or nucleoli visible (H&E x575).

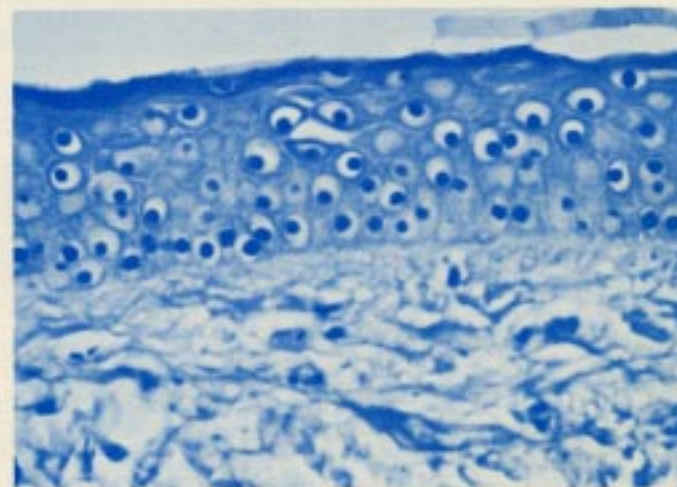


FIGURE 4:
Effects of xylene: Severe nuclear shrinkage, no detail of nuclear chromatin, and obscured nucleoli (H&E x575).

use of tissue processors which contain heat, vacuum and oscillation. This equipment, which incorporates gentle heat (38-40° C), vacuum (15" Hg), and oscillation, accelerates fixation, dehydration, clearing, and impregnation of tissue specimens.

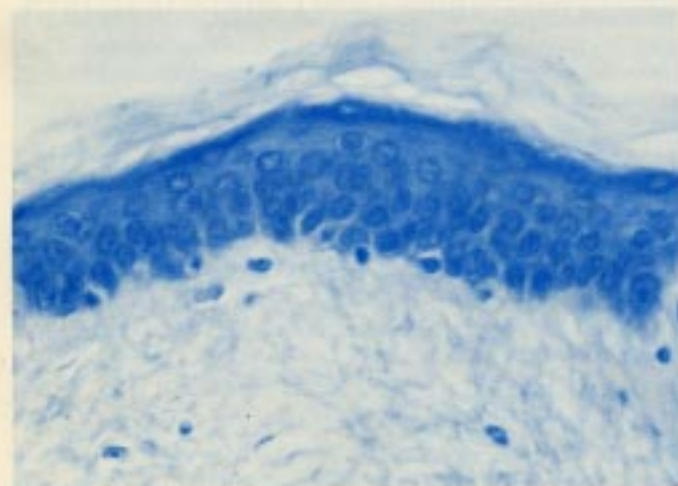
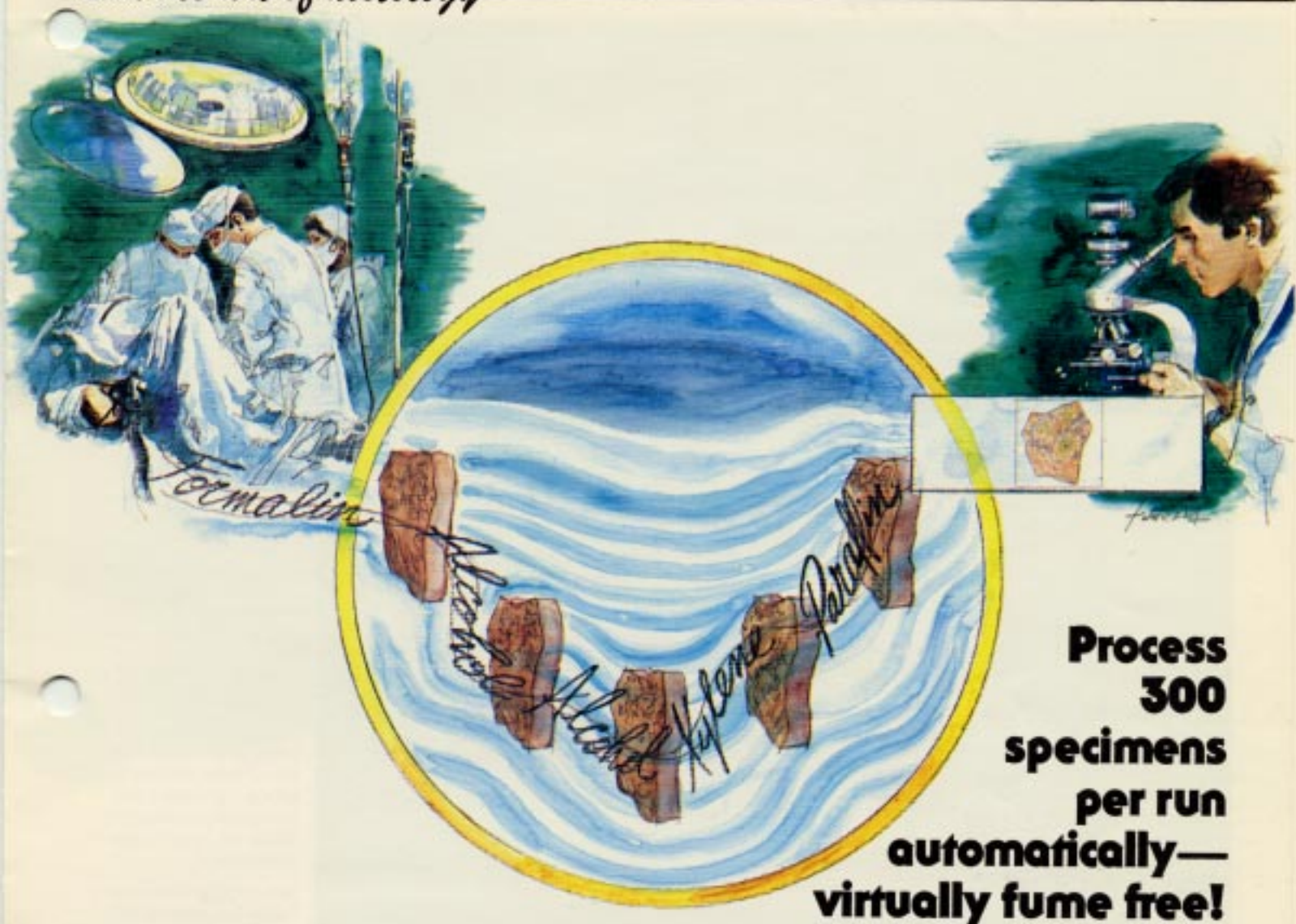


FIGURE 1:
Specimen fixed in phosphate-buffered 10% neutral formalin. Excellent preservation of cellular structures (H&E x575).

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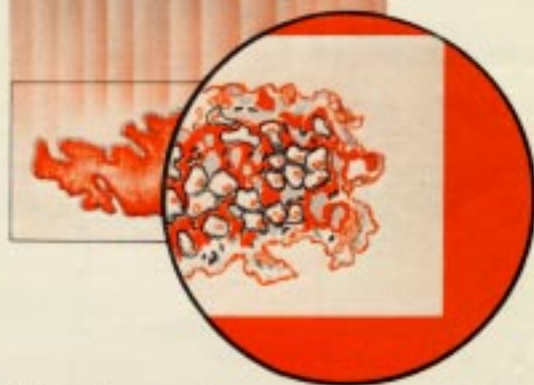
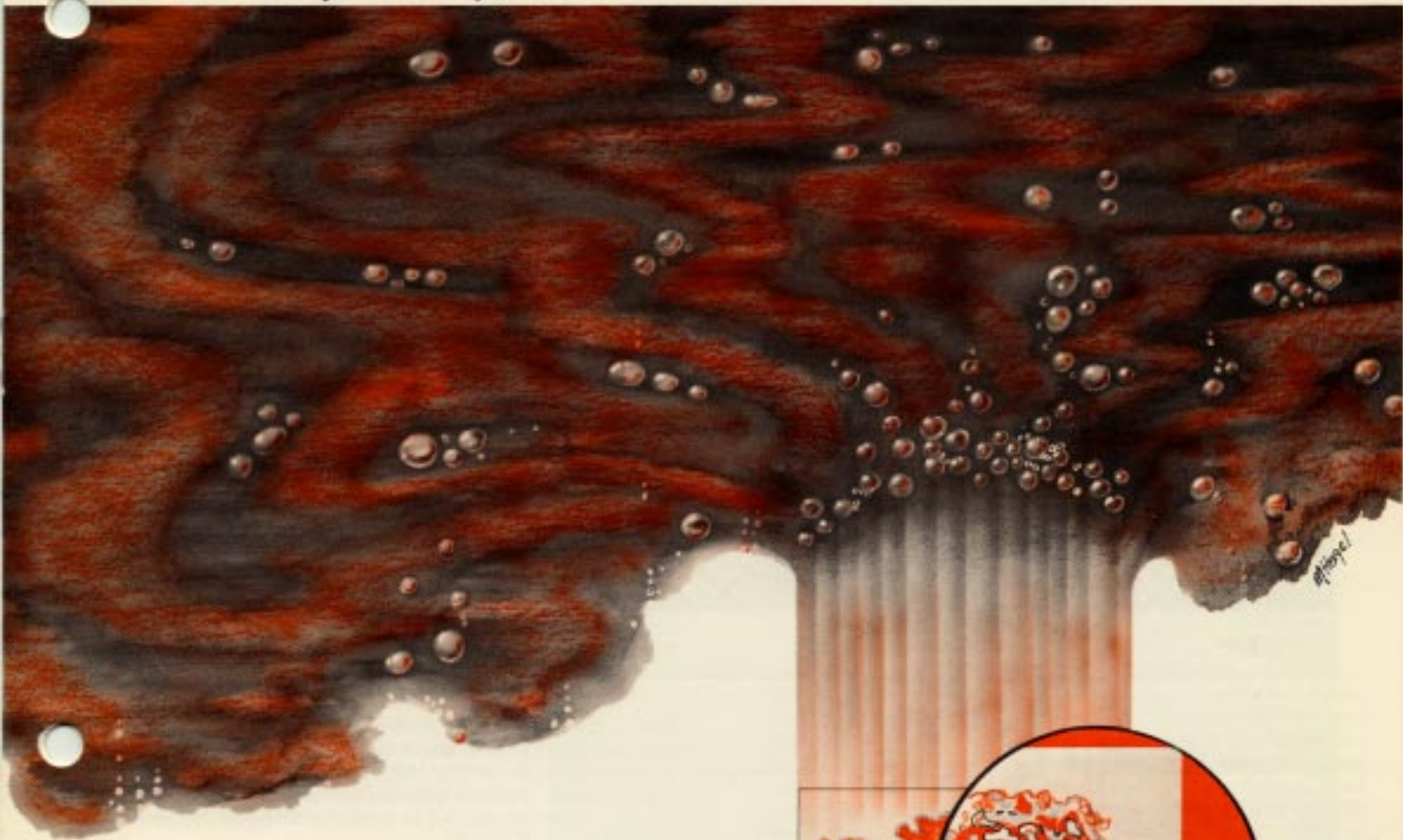
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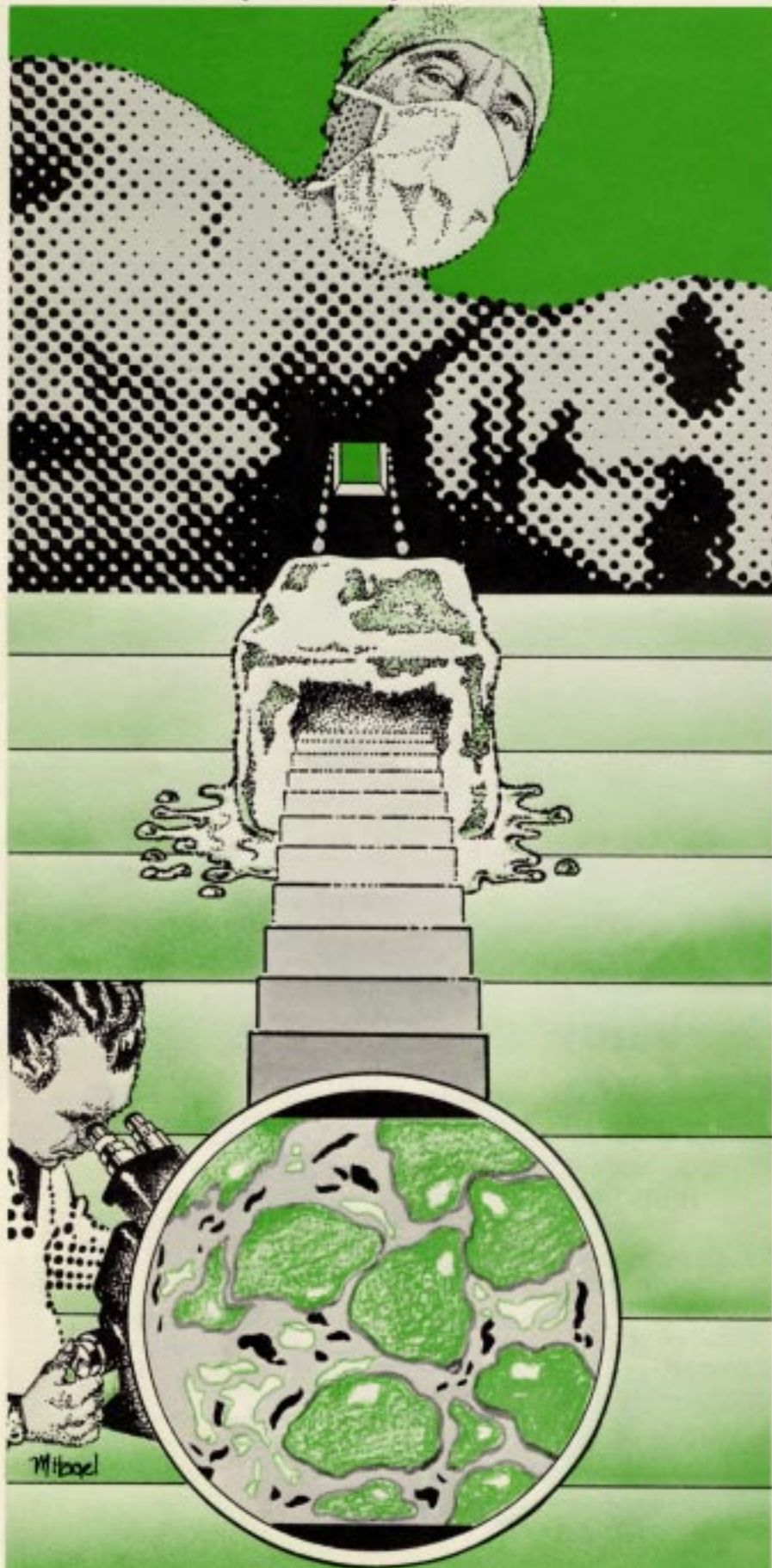
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The editor wishes to solicit information, questions, and articles relating to histotechnology. Submit these to: Lee G. Luna, Editor, Histo-Logic, P.O. Box 36, Lanham, Maryland 20801. Articles, photographs, etc., will not be returned unless requested in writing when they are submitted.