A Simplified System for Studying Digestive Function and Responses

By T. DANIEL KIMBROUGH and GERALD C. LLEWELLYN

Students of biology or physiology are continually raising questions pertaining to the responses of living tissue to various chemical compounds. Their interest often stems from current news releases and cautions from the U.S. Food and Drug Administration about food additives, food contaminants, pharmaceuticals, illicit drugs, and carcinogenic compounds. Some rather simple but interesting experiments can be designed by using sections of the intestine taken from various small animals. Experiments such as these could provide potential answers for the students' experimental hypotheses as well as introducing a sensitive laboratory testing device.

Principles of Motility

Smooth-muscle motility within the alimentary canal of an animal is a virtually endless process. In addition to peristalsis, the basic propulsive movements of the intestinal tract have been described; they include segmenting, pendular, rippling, and villi pumping activities. It is the combined function of these various types of motility that is responsible for the mixing movements of the alimentary canal (Bishop *et al.*, 1966).

In order to achieve a better understanding of the functional types of gastrointestinal motility, some knowledge of the anatomy of the intestine is necessary. Fig. 1 is a typical section of the gastrointestinal tract, illustrating the following layers from the outside inward: the serosa, a longitudinal muscle layer, a circular muscle layer, the submucosa, and the mucosa. In addition there is shown an intramural nerve plexus composed principally of two layers of neurons and appropriate connecting fibers. The outer layer is called the myenteric plexus; the inner layer is called the submucosal plexus, or Meissner's plexus.

The anatomic basis for gastrointestinal motility is the formation of a functional syncytium by each layer of intestinal smooth muscle. A syncytial arrangement is one in which the individual muscle fibers lie in such close contact with each other that stimulatory impulses started in any part of the muscle tend to spread to contiguous tissue.

Two kinds of contractions are observed to occur in gastrointestinal smooth muscle: tonic and rhythmic. Tonic contraction is long-lasting—sometimes continuing for hours with varying intensity. The

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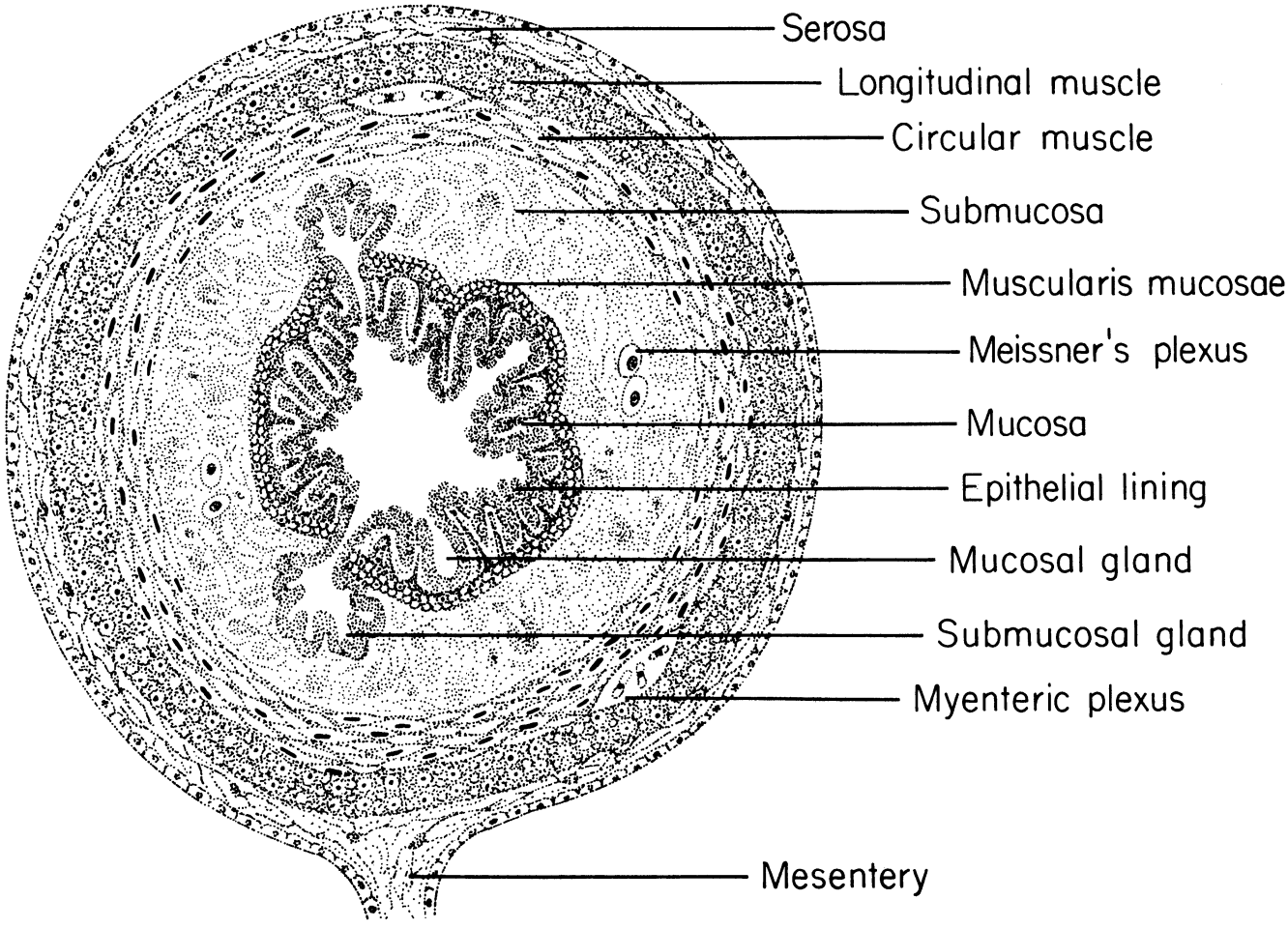

Fig. 1. Typical cross-section of the mammalian duodenum.

amount of steady pressure in the intestine is dependent on the degree of tonic contraction in each segment. Mean rhythmic contractions in vertebrate animals occur, usually at a rate of 10 to 17 times a minute. Segmenting movements of the circular muscle of the gut exemplify rhythmic activity. Variations in rates of intestinal contractility are believed to result both from the different kinds of metabolic activities occurring in smooth muscle and from the effect of nerve fibers and hormones. The overall effect of both tonic and rhythmic contractions, as influenced by such factors, is to maintain the intestinal contents in motion (Guyton, 1971).

Table 1. Composition of Tyrode's solution.

8.00 gm sodium chloride (NaCl)
0.2 gm potassium chloride (KCl)
0.2 gm calcium chloride ($CaCl_2$), anhydrous
1.0 gm sodium bicarbonate ($HaHCO_3$)
0.1 gm magnesium chloride ($MgCl_2$)
0.05 gm dibasic sodium phosphate (Na_2HPO_4)

—in 1 l of distilled water. $NaHCO_3$ must be completely dissolved before $CaCl_2$ is added. Add 0.1 gm glucose to every 100 ml before using (Armstrong, 1969).

The body of an animal contains three different kinds of muscle: skeletal, cardiac, and smooth. Intestinal musculature is of the smooth variety; that is, the striations that characterize the other types are not visible when this muscle is viewed with the light microscope. Nevertheless, electron microscopy has revealed that such striations, although quite small, do exist; furthermore, investigators have found that the basic physiology of contraction for smooth muscle is fundamentally similar to that of other types. That is to say, muscle contracts when certain protein filaments slide past one another or interdigitate. The initiation of this filament-sliding involves the interaction of several well-known components, the most obvious of which are the muscle proteins (actin and myosin), the T-tubular system, the sarcoplasmic reticulum, sodium and calcium ions, and ATP. The contractile mechanism may be briefly described as follows: When an impulse is directed

to cause muscle to contract, a chemical known as acetylocholine usually is released at the nerve–muscle junction (myoneural junction). This chemical, acting on the membrane of the muscle fiber, allows sodium ions to enter into membrane pores. The rapid influx of sodium ions then creates an endplate potential, which results in the excitation of a muscle fiber in a very short period of time. For example, after stimulating a 10-cm muscle fiber the impulse will reach both of its ends in about 1/100 second. Meanwhile, following the excitation of the muscle fiber, the action potential not only travels over the surface of the membrane but also penetrates deeply into the fiber by way of the T-tubules (fig. 2). As it flows throughout this canal system the impulse causes the release of calcium ions from storage in the sarcoplasmic reticulum. Following their release into the fluids surrounding the myofibrils, these ions flow via the same canals to the site of contraction. There they function in the activation of the enzyme ATP-ase, which serves in turn in the release of free energy from ATP (Bohr, 1971). This is the energy that must be made available for the breakage of the bonds, or "cross-bridges," which previously had prevented the interdigitation of the muscle filaments. With the cross-bridges thus temporarily removed, the filaments slide freely; that is, the muscle contracts (fig. 3). After a few thousandths of a second the calcium ions return to their storage sites, and once more the cross-bridges form. The muscle then relaxes (Guyton, 1969; Huxley, 1965).

In spite of the similarity of its contractile mechanism to those of other types of muscle, certain characteristics distinguish smooth muscle from skeletal and cardiac muscle and uniquely fit it for function in the alimentary canal. One of these is extensibility.

Table 2. Intestinal-motility responses to selected agents. Relative responses are based on a predetermined concentration.

Agent perfused	*Relative response*
Neostigmine	Strongly stimulatory
Nicotine	Moderately stimulatory
Acetylcholine	Mildly stimulatory
(Untreated)	(Natural)
Ethanol	Mildly inhibitory
Noradrenalin	Moderately inhibitory
Adrenalin	Strongly inhibitory

This property provides the basis for peristaltic and segmenting activity and for the slow rhythmic and tonic contractility that is observed only in the intestinal tract (Bülbring *et al.*, 1970).

Laboratory Applications

By understanding the basic nature and properties of smooth muscle one can prepare himself for designing the experiments suggested here. These experiments, if properly conducted, will result in an even greater appreciation of how an animal processes its food and how it may use certain naturally occurring substances to alleviate conditions resulting from improper dietary habits. This might be illustrated by considering the influence of the excess of magnesium ions brought into the alimentary canal through the consumption of milk of magnesia, a popular laxative. In this particular case the excess calcium ions increase the influx of liquids into the lumen of the gut.

After the student has encountered the basic anatomy and related physiology of intestinal tissue he is

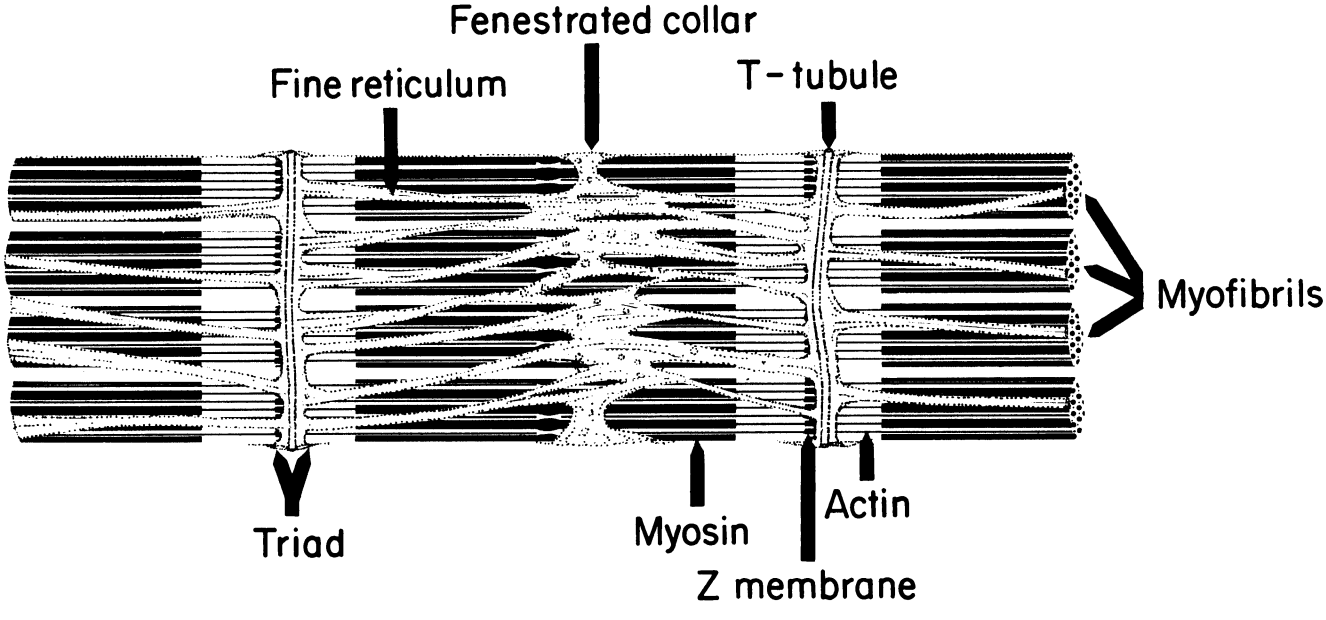

Fig. 2. Organization of the T-tubules within the basic contractile unit.

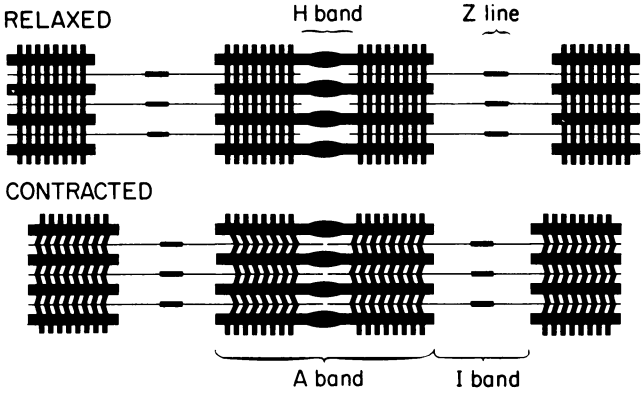

Fig. 3. Sliding of the actin and myosin filaments within the basic contractile unit.

prepared to explore some avenues of research. The use of this procedure as an introductory demonstration, an individual project, or a class laboratory will depend on the teacher. The procedure has been tested in high school and college introductory-physiology laboratories; as a result of these tests we recommend that groups of students perform the experiment.

Several recent investigations have indicated that an intimate relationship exists between the hormones and the nerves of the intestinal tract. It has been shown, for example, that serotonin (5-hydroxytryptamine), which is stored within the enterochromaffin cells of the gut mucosa, is released in response to nervous stimuli (Gershon *et al.*, 1965). On being released, the serotonin apparently impinges upon receptors in the submucosal and myenteric plexuses, respectively; and this results in the neurohormonal increase in gut motility. The wide range of responses of the intestine to such stimuli have led to the use of motility measurement as an ultrasensitive tissue indicator for a variety of studies. Through the use of isolated strips of gut one may obtain a better understanding of the nature of numerous agents that find their way into the alimentary canal and somehow stimulate the neuroendocrine mechanism.

Procedure

The physical design for the proposed work includes an *in vitro* procedure and a widely available laboratory instrument, the kymograph. If this instrument is available and if the appropriate animals—rats, rabbits, hamsters, gerbils, or frogs—have been chosen for the demonstration of intestinal rate responses to various experimental parameters, the following procedures may be initiated:

1. Humanely sacrifice the animal by decapitation with a sharp, heavy cutting instrument, such as a pair of shears.

2. Use a sharp pair of scissors to make a midline, peritoneal incision. Excise a 3-cm strip of duodenum. This tissue is an excellent choice for motility study because it contains the neurohormone serotonin, which seems to increase the intestinal motility in the normal animal (see above). Serotonin and other hormones account for up to 75% of the contractile activity occurring within the alimentary canal.

3. Place the gut strip in a small beaker of warmed (37.5 C) Tyrode's solution (table 1) and gently swirl to flush out the digested contents. Maintain the original temperature of the fluid in the beaker until the perfusion chamber has been prepared (fig. 4). Time lapse between sacrifice and perfusion should not exceed five minutes. The perfusion chamber consists of oxygenated test-tubes of perfusate (Tyrode's solution) immersed in a constant-temperature water-bath. The gut strip is then hooked at both ends with pins (insect pins bent like a fishhook), which connect below to the J-shaped arm of a glass aeration tube and above to a point about midway along the muscle lever. A counterweight, such as a large paper clip, is then fastened near the inscribing end of the lever in order to maintain tautness in the gut-strip assembly.

4. The following precautionary measures are suggested for the success of the procedure thus far:

a. Keep the tissue warm and moist with Ty-

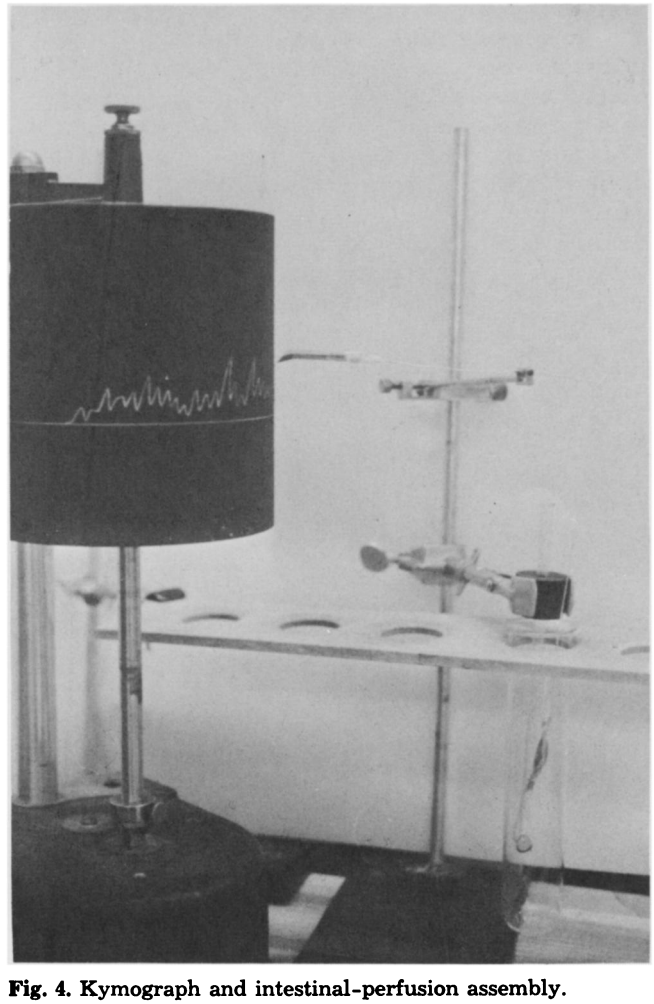

Fig. 4. Kymograph and intestinal-perfusion assembly.

rode's solution. Avoid undue handling and stretching of the gut strip.

b. Keep the oxygen bubbling at a constant rate through the J-tube. If oxygen is not available, aeration may be supplied by means of an inexpensive aquarium pump having an in-line filter.

c. Maintain a constant waterbath temperature. A small, rectangular aquarium may be used for the bath, and one or more aquarium heaters may be used to assure the proper temperature setting.

5. The perfusion assembly consists of (i) the muscle lever with writing point (ink-writing equipment may be used); (ii) the gut strip assembly; and (iii) the perfusion chamber, which consists of a wide-mouthed test-tube (200 ml capacity) filled with approximately 150 ml of oxygenated Tyrode's solution and maintained at constant temperature.

6. Add 1 g of glucose per liter of Tyrode's solution (0.1%) as a nutrient. Submerge the gut-strip preparation and allow it to acclimate for 10 to 15 minutes before beginning the recording.

7. Set up the previously prepared kymograph drum so that the writing point of the muscle lever inscribes a distinct marking on the paper. A slow drum-speed should be used. Next, inscribe a baseline around the drum to provide a reference guide. For relatively short periods of recording, a single drum surface should suffice; if longer periods of recording are necessary (for example, 30 minutes) the use of a dual kymograph system is suggested. This system, beltlike in nature, consists of two kymographs with both drums driven by the motor of the first. They may be spaced over distances ranging from 3 to 6 m. Self-adhering segments of prepared kymograph paper, reinforced with tape, may be joined to construct the belt.

8. Start the recording by first obtaining a 10-minute measurement of the contractions of a control (equilibrated) gut-strip. Thereafter, a variety of tests and responses may be undertaken when selected measurements of agents chosen for study are placed in the perfusate. The following examples are possible experiments:

a. Determination of degrees of response to concentration variances; for example, the study of changes in amplitude of response to Cu^{++}, Pb^{++}, Mg^{++}, and Hg^{++}.

b. Instigation of a study of air pollution through the infusion of certain noxious gases into the perfusion assembly. Such gases as nitrogen, ammonia, sulfur dioxide, carbon dioxide, and carbon monoxide might be used in lieu of oxygen or air. Such measurements might be applied to the construction of a "response index," which could well lend itself to timely studies related to air pollution. Artificial smog, if available, could be used.

c. Responses to household chemicals. These could include detergents (phosphate, non-phosphate and enzymatic), meat tenderizers, spices, diuretic agents (coffee, tea, etc.), vinegar (acid), and carbonate salts (base). Of possible interest regarding acid and base compounds tested is the observation that digestive juices of the duodenum undergo radical changes from about pH 2 (gastric outflow) to a pancreatic enzyme-mediated change of about pH 8. The changes of pH would in turn be reflected by changes in gut motility.

d. Response to nonprescription pharmaceuticals, such as aspirin and buffered "pain relievers"; decongestants and antihistamine compounds; and tranquilizers, sleep-inducing compounds, and stimulants.

e. Response to pharmacologically active compounds, such as adrenalin and noradrenalin; acetylcholine; ethanol; nicotine; phisostigmine or neostigmine; curare (3%); strychnine; insecticides (organophosphates, arsenicals, chlorinated hydrocarbons); rodenticides; and herbicides. These experimental compounds may be studied further through the evaluation of their "synergistic" effects, which could result in potentiated, antagonistic, or neutralizing effects. Such would involve concurrent infusion of matched pairs of the pharmacologically active compounds listed, using the gut strip as a marker.

Data Compilation and Interpretation

The kymograph records (kymograms) may be evaluated as to (i) contraction frequency (peaks per minute), (ii) amplitude (peak heights, maximum and minimum), and (iii) composite measurements of the area, obtained by tracing (outlining the perimeter) peaks with ordinary sewing thread or (iv) by weighing selected areas of contractility that have been carefully timed, enclosed, and trimmed. The paper used should be of uniform thickness.

Discussion

The technique described in this article provides several ways of gathering data; at the same time it suggests a number of experiments with some common, easily obtainable compounds of current interest. Some of these have been chosen because of their possible detrimental effects on the public health or the environment. Through the use of the intestinal-strip perfusion assembly the student investigator or class is given a highly sensitive device for assaying relative toxicity and responses of compounds. This can lead to the arrangement of these compounds in the form of indices of response. Such a rating system could be constructed to indicate high-to-low ranges for both stimulatory and inhibitory responses. A sample response index is given in table 2 (Kimbrough and Sharpley, 1971).

(Concluded on p. 163)

Letters to the Editor

• Brief letters—one or two pages—are more likely to be printed than are long ones, which may be cut.

Student Likes Water-Pollution Article

I read with interest the article "Teachers and Students Write a Curriculum on Water Pollution" (*ABT* 33 [4]: 211-213 f.). I was just introduced recently to our nation's water problems in my biology class here at school. Naturally, due to the various news media that we have, Americans are becoming more aware of the environmental crisis that we have to face.

The article concerning the Tilton School project in New Hampshire was interesting. I liked the idea of the students as well as the teachers participating together on the project. But I think it is a mistake in education to bring the pollution problem to the attention of the young people at the high school age. I think that's too late. Wouldn't it be more beneficial to introduce the nation's environmental problems to the children at the elementary school age? The children today should be shown the mistakes that the adults have made, in the hope that they will not make the same mistakes that their parents did.

You brought out how the Tilton activity had originated in Cleveland in 1967. I am sure that there are probably other projects going on throughout the country dealing with water pollution and perhaps offering a solution. But here in northeastern Ohio the water pollution problem really hits home with the contamination of Lake Erie and the pathetic condition of the Mahoning River here in Youngstown, Ohio.

I hope that the curriculum on water pollution will help the teachers to educate the children in the schools so that in the future we can remedy this national problem.

Thank you for that interesting article.

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William H. Schlesinger comments:

Thomas Gross's comments are most appropriate. The earlier a youngster is exposed to environmental education the more perceptive will be his appreciation of environmental problems. With this in mind, the Tilton School curriculum was tested with elementary-school teacher and student participants during the National Association of Independent Schools' training session in August 1970. Being moderately successful even in a form written primarily for high school use, the program indicated that elementary-school students could be exposed to environmental education to provide a background for later, more detailed study. The Tilton School curriculum guide, entitled *A Curriculum Activities Guide to Water Pollution and Environmental Studies,* is now available from the Institute for Environmental Education, 2803 Scarborough Road, Cleveland Heights, Ohio 44118.

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In general, this procedure requires a minimum of equipment and materials. Furthermore, with the exception of the kymograph (which in fact can also be home-constructed) all items are inexpensive and are widely available to the student.

Acknowledgement.—Elaine H. Mullen prepared fig. 1, 2, and 3.

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MORE RECREATION TRAILS

The U.S. Interior Dept. recently designated 27 new National Recreation Trails to be added to the National Trails System. The trails—ranging in length from one-quarter mile to 30 miles—are located primarily near urban population centers where they offer outdoor recreation to hikers, bicyclists, horseback riders, naturalists, and the handicapped. The 1968 National Trails System Act provides chances for state, local, and privately owned trails to become part of the system pending approval by the Interior secretary.