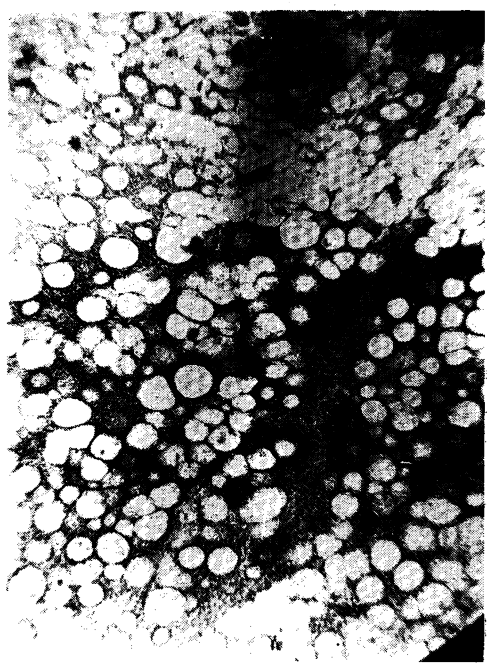

FIG. 3. Fat tissue surrounding ovary from obese female 8 months old, weight 42 g. Anomalous areas of darkly stained cells are widespread.

similar suggestion has been made recently by Ingle (*10*). This scheme is supported by observations of adiposity effects in preadolescent children, who commonly show delayed puberty. Simple weight reduction by dietary means results in the fairly prompt establishment of puberty (*3*). Obesity is also associated with other endocrine anomalies in man, some of which have a hereditary basis (*9*). It is conceivable that the path of gene action follows the scheme obesity←←A^y→→sterility, although this sequence is improbable in view of the observations discussed above. Pleiotropic effects have been observed in various organisms, but some of them have been shown to be spurious (*11*).

One might expect obese male mice also to show gonadal anomalies. This has been observed to some extent in rats made obese by overfeeding or by underactivity (*10*). Yellow males examined in the present study were only slightly obese, and their testes showed normal spermatogenic stages. In addition, spermatozoa and interstitial cells appeared normal when compared to black mice. It would have been desirable to examine the testes of very obese males, but because of technical difficulties, this was not done. More exact data on the fertility of these obese males, as well as inbred yellow mice, would also be desirable. Attempts to influence obesity and sterility by means of endocrine preparations have not yet been made.

Obesity in mice carrying the yellow gene is caused by an increase in food intake and less physical activity. These two effects of the gene are not primary ones and are mediated by metabolic dysfunctions involving the endocrine system. Data presented here support the hypothesis that obesity resulting from this hormonal disorder upsets normal ovarian function, causing sterility. The possibility that the ovary itself is the site of the hormonal disorder causing obesity is not excluded by this hypothesis.

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Automatic Microtome

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This paper will describe a new instrument and method for automatically cutting and mounting thin sections of embedded biological specimens.

The manual method now used has several disadvantages. It is laborious, since the cut sections have to be mounted manually; the pressure of the knife on the soft impregnating material causes distortion, sometimes as much as 10%; the sections are not registered for rapid examination of corresponding areas. These disadvantages are inherent in the method of first cutting sections from the top of an impregnated block and then mounting them. Convenience and improvement of register should therefore be obtained by a method which, in effect, mounts the sections first and slices them off afterward.

The automatic microtome does in fact combine the operations of mounting and slicing in such a manner that the specimen tissue is supported by the mounting film during the slicing. As a result there is obtained

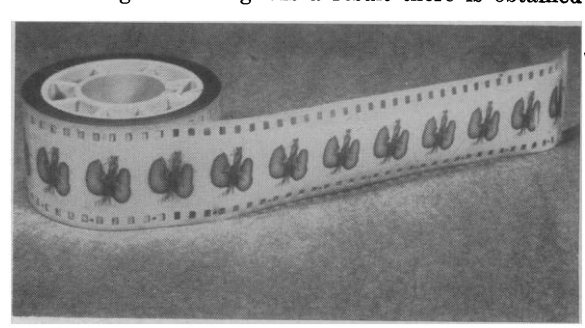

FIG. 1. Film .003" thick showing mounted sections of 90-mm pig kidneys.

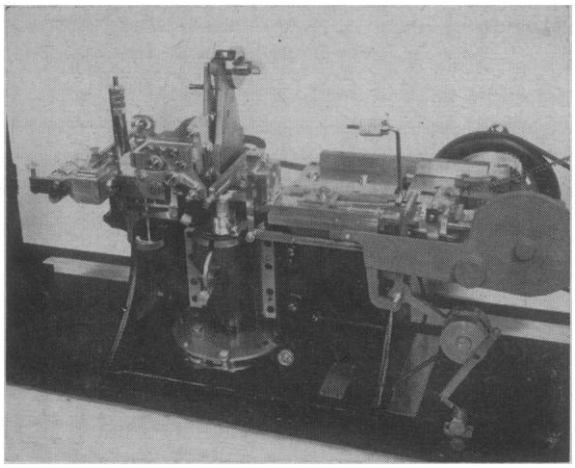

FIG. 2. General view of the automatic microtome.

a strip of film base with the sections mounted at intervals as shown in Fig. 1. Registration is precise with respect to the film perforations within the tolerances common to motion-picture technique. Since the section is firmly supported while being cut, distortion has been practically eliminated.

Fig. 2 shows a general view of the automatic microtome. A Spencer instrument was used as a basis for its development. The added features consist of a film-handling mechanism and a device for pressing the film onto the block and guiding and supporting the film during the slicing stroke. The microtome is motor-driven at 10 sections/min, and controls are by cams so that all operations are automatic.

The film-handling mechanism follows motion-picture techniques except for departures necessitated by the use of thin base and relatively high tension. The film base used is of standard 35-mm width, but is thinner than standard.[1] With film .0035″ thick it has been found possible to cut specimen sections down to 10 μ successfully. Film base .002″ thick will permit thinner sections but is more difficult to handle. With thin base film of this sort, care is required in the construction of the film-advancing mechanism. This is of the usual claw type, with lateral registration obtained from the edge of the film. It has been found necessary to hold the film under considerable constant tension to ensure registration. Clamps hold the film during the slicing stroke. The claw mechanism operates on several perforations to avoid distortion at this point. Its stroke is limited by an adjustable stop in order to ensure longitudinal registration; the usual method of securing this by pins is not satisfactory with thin film in the presence of tension.

[1] Eastman Kodak Company and E. I. du Pont de Nemours & Company have kindly supplied special film.

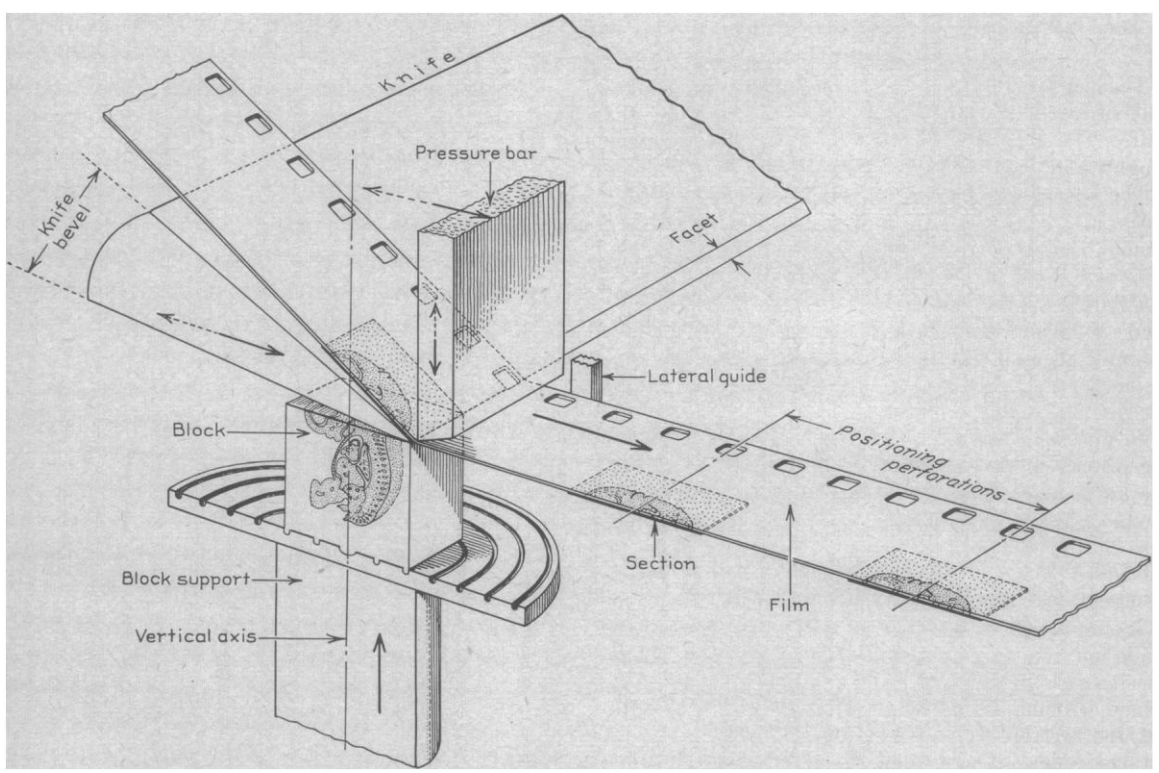

FIG. 3. Scheme for automatically applying serial sections to 35-mm perforated cellulose acetate film. This diagrammatic median view shows the block support carrying an embedded embryo, with the knife completing a cut with the pressure bar still in the functioning position. Forward motion is indicated by a solid arrow on the film, and reciprocal motions are indicated by solid–broken-line arrows. The lateral film guide moves with the pressure bar but is carried on a separate mount.

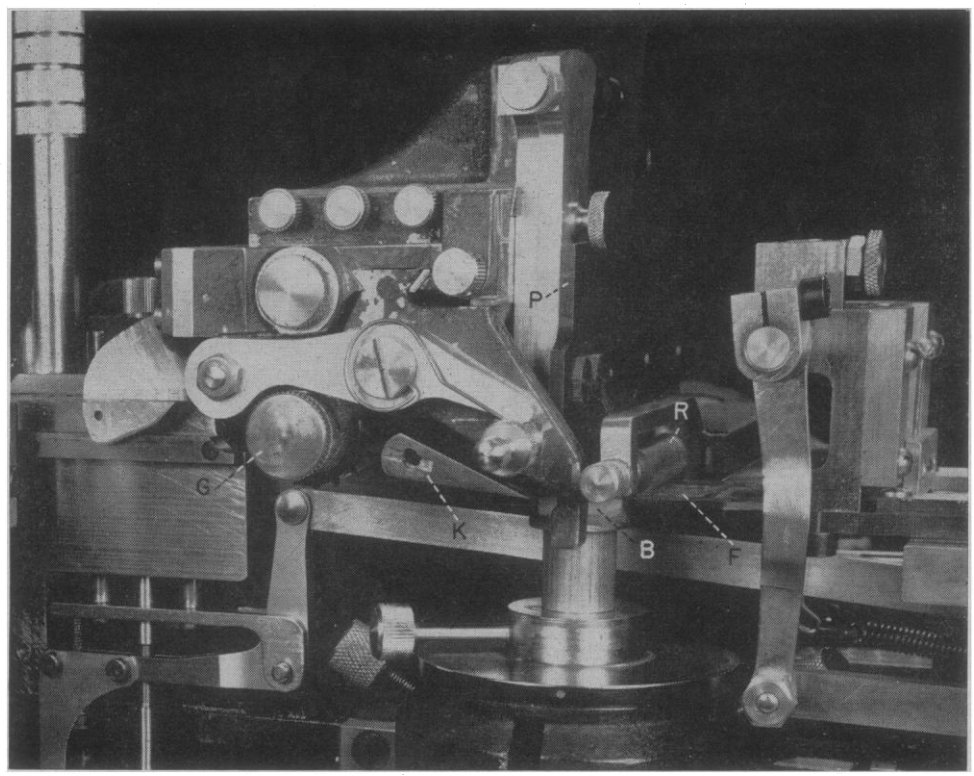

FIG. 4. View of construction of the automatic microtome. *B*, specimen block; *F*, film; *G*, guide roller; *K*, knife; *P*, presser blade; *R*, roller.

The heart of the device is the mechanism that guides and supports the film during the operation of the knife. The diagram in Fig. 3 shows the relations sought, and Fig. 4 gives a view of the construction.

The specimen, usually double-embedded to form a block, is at *B* in Fig. 4. The film *F*, when the knife is remote, is stretched over the surface of this block and in light contact with it. In this position it is advanced to a new frame and clamped. The roller *R*, the presser blade *P*, and knife *K*, and the guide roller *G* are mounted together on the knife carriage. There is provision for holding the tension in the film constant as the carriage moves forward.

In operation, the knife carriage advances carrying the presser blade across the block above the film and the knife blade through the block under the film. As the block is sliced the presser blade controls the curvature of the film at the point of cutting the double-embedded matrix and tissue. Full bonding occurs practically simultaneously with the cutting. The roller *R* is employed to keep the film clear of the presser blade on its return stroke. With double-embedded specimens and acetate film base, good adhesion is obtained without added adhesives. After the section is cut, the presser blade is raised and the knife carriage returns, whereupon the film is advanced and clamped for the next stroke.

The position of *P* with respect to the knife edge needs careful adjustment. It must be brought parallel to the knife edge by two adjustments and set at a proper distance in advance of the knife. The pressure it exerts is adjustable, and there is a stop to prevent it from descending too far. The positions of the knife and the guide roller and the tension are also adjustable. These are all fairly critical adjustments. When they are once made, the device will operate indefinitely to give satisfactory sections of a given thickness.[2]

The film with its mounted sections is surprisingly rugged. It has been found desirable, however, to wind a thin protecting film with the working film as it goes onto the take-up spool.

Sections mounted on film in this manner are convenient to use. Treatment, such as staining, may be done while handling the film, by the usual photographic equipment. In this connection it will undoubtedly be desirable to use film base material other than cellulose acetate for some processing purposes. Projection or examination under the microscope is facilitated, since ordinary 35-mm film-handling equipment can be used to bring successive sections into register. Copies may readily be made with ordinary motion-picture equipment. The basic principle of the microtome—that is, the support of the section during the period when it is being cut—has been applied to

[2] The first model of the machine was built by W. R. Horsfield, now of Bermuda. My thanks are especially due to Osborne O. Heard, of the Department of Embryology, who carried out the difficult process of removing faults from the machine, and succeeded in producing excellent sections. W. F. Steiner and L. A. Horton, of the Department of Terrestrial Magnetism, made final alterations necessary for fully precise registration.

frozen sectioning techniques. Certain other manipulations, to be described in later papers, are also facilitated.

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Histochemical Demonstration of 5-Nucleotidase Activity in Cell Nuclei[1]

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Evidence has been brought forward recently which seriously questions the value of histochemical methods for the localization of alkaline (*1*) and acid (*2*) phosphatase within cellular structures. In the case of alkaline phosphatase, nuclear staining is seen in many tissues with the Gomori (*3*)–Takamatsu (*4*) technique, but not with the azo-dye method (*5, 6*). All or part of this nuclear activity may, however, be due to unspecific staining, since cell nuclei absorb phosphatase, which readily diffuses from tissue components with high activity, as well as calcium phosphate which has been split off the substrate in the Gomori–Takamatsu method (*1, 7–9*).

In the present communication, evidence is presented that an enzyme attacking adenosine-5-phosphoric acid (muscle adenylic acid), first found by Reis (*10, 11*) in various tissue extracts, can be localized not only in the cytoplasm but also in the nuclei of some tissues. Such a 5-nucleotidase, differing from the unspecific phospomonoesterase, has been previously demonstrated histochemically (*12, 13*).

Thin slices of tissue were fixed in ice-cold acetone for 24 hr, then dehydrated for 24 hr in absolute alcohol or cedar wood oil and cleared in 2 changes of xylol for 1 hr each. Paraffin sections were cut at 5 μ and incubated in the substrate at 37° C. The substrate contained 1.44×10^{-3} *M* adenosine-5-phosphoric acid,[2] 8.0×10^{-2} *M* calcium chloride, 1×10^{-2} *M* magnesium sulfate, and 2×10^{-1} *M* 2-amino-2-methyl-1,3 propanediol buffer at pH 8 (*14*). The sites of formed calcium phosphate were visualized by treatment with cobalt nitrate followed by diluted ammonium sulfide. The adenosine-5-phosphoric acid was added to the substrate mixture as dry powder (50 mg/100 cc). In preliminary experiments this amount was found to be the smallest that gave satisfactory results. The concentration of calcium chloride was within the range suggested recently by Gomori (*12, 15*). Gomori pointed out that a high concentration of calcium would substantially decrease the solubility of the formed calcium phosphate and thus prevent unspecific staining caused by secondary absorption of the dissolved calcium phosphate from the substrate.

[1] This work was supported by a grant from the Damon Runyon Memorial Fund for Cancer Research, Inc.

[2] The adenosine-5-phosphoric acid was obtained from Sigma Chemical Co., St. Louis, Mo., and from Schwarz Laboratories, Inc., New York. Identical results were obtained with both preparations.

Myometrium of fresh surgically removed uteri and aorta of recently killed rabbits was used. In both tissues the usual technique for demonstrating alkaline phosphatase with glycerophosphate as substrate buffered at either pH 8 or pH 9 revealed only staining of capillaries in the myometrium and of the endothelial layer in the aorta. No other structures showed activity even if the incubation time was extended to 12 hr.

5-Nucleotidase activity was demonstrable in the cytoplasm of smooth muscle fibers, interstitial fibroblasts, and the cells composing the wall of the myometrial arteries after 3 min incubation and increased markedly in the following minutes. Staining was always diffuse and even. The coating of sections with celloidin did not alter the results. For comparison, frozen sections cut at 10–15 μ were prepared from fresh, unfixed uteri, as well as from material which had been fixed for 24 hr in ice-cold acetone. In these preparations enzymatic activity in cytoplasm and nuclei could be recognized after 2–3 min and was quite distinct after 4 min. The distribution of histochemically demonstrable enzymatic activity was identical with that seen in paraffin sections. Excellent preparations for microscopic study were obtained in paraffin sections after an incubation period of 10–60 min. Within the nuclei the nuclear membrane, nucleoli and chromatin particles were stained. Within the cytoplasm of both the smooth muscle cells and the fibroblasts fine dark staining fibrils could be recognized (Fig. 1). If the incubation period was extended over several hours, all cellular details were obliterated by the heavy deposit of calcium phosphate after its visualization as dark cobalt sulfide.

FIG. 1. Myometrium from human uterus. Paraffin section incubated for 10 min. Note prominent nuclear staining and fibrillar structure of cytoplasm. × 500.

With the rabbit aorta activity was demonstrable in 6 min and was quite distinct after 10 min incubation in paraffin sections (Fig. 2). Nuclear staining in smooth muscle fibers and fibroblasts was as prominent as in the myometrium. The cytoplasm of these cells showed a comparable amount of activity, whereas the elastic membranes remained completely unstained.

In order to establish the optimal pH range for the