

7-day-old axillary implants, we have never seen regrowth of tumor tissue at the site of extirpation or in remote sites, although care must be exercised in making the original implant so that no tumor tissue fragments are deposited along the route of trocar entry. Implants of mouse sarcoma 180 older than 7 days become progressively more difficult to remove by this or any other known method, for such growths have usually undergone an irretrievable infiltration into the body wall.

References

1. GREENSTEIN, J. P., and ANDERVONT, H. B. *J. Natl. Cancer Inst.*, **2**, 403 (1942).
2. LUMSDEN, T. *Lancet*, **2**, 542 (1925).
3. ZAHL, P. A. *J. Natl. Cancer Inst.*, **11**, 279 (1950).

Tubal Malignancy—A Method for Collecting Specimens for Cytologic Study

K. Sheldon MacLean

135 East 65th Street, New York

Exfoliative cytology of the female genital tract, since Papanicolaou's monumental work, has become one of the most popular and probably the most important diagnostic procedure for the early detection of cancer. As the diagnostic criteria for the malignant cell become better defined, the percentage of positive diagnoses increases, missed diagnoses become rarer, and false positive diagnoses are almost completely eliminated. The ready accessibility of the cervical canal through which exfoliative cells from the uterine cavity pass (and in some cases from the appendages) renders cytologic investigation a relatively simple procedure.

Unfortunately, however, in cases of tubal malignancy, fewer cells are expected to reach the cervical canal, and consequently their detection becomes difficult and impractical, unless a way is found to collect and concentrate the material over a certain period of time, without added difficulties and without discomfort to the patient. This has been done as follows:

After cleansing the vaginal tract, a plastic cervical cap¹ of proper size is fitted over the cervix. The cap is removed 24 hr later and slides are prepared from the collected material. If the volume of secretion is excessive, the material may be concentrated by centrifuging or by permitting it to settle. Longer periods were tried—e.g., 48, 60, and 72 hr—and after such intervals a larger amount of secretion was collected, as anticipated. Because of autolysis, however, the stain characteristics and clarity of the detailed cellular structure were impaired, making the slides unsuitable for proper interpretation. In some cases the patient complained of physical discomfort and offensive odors if the cap was left longer than 24 hr. It is therefore recommended that the cap be removed in 24 hr, and slides prepared and processed immediately.

¹ Manufactured by the Ortho Pharmaceutical Corporation for use as a contraceptive.

The use of the cap has made possible a positive diagnosis of tubal carcinoma in a clinically unsuspected case in which there was no palpable mass. The diagnosis was subsequently confirmed by operation.² The procedure is now in routine use in the writer's practice. Results will be reported later.

² The case will be reported in detail later.

Action of Genes Affecting Secondary Sex Ratio in Man

Marianne E. Bernstein¹

Istituto di Statistiche, Rome, Italy

C. Gini in Italy and, later, E. Slater in England, have shown conclusively that the tendency in a family to produce offspring of one sex only or primarily one sex, is hereditary. On a large set of American families this author has shown also that there is a decided excess of sibships of only sons or only daughters. Since all these findings point so strongly toward genetic control of the sex ratio, a study was made as to how these genes act. Statistical and experimental investigation led us to advance a theory that the "sex ratio genes" act through the endocrine system, especially the sex hormones. Fathers suffering from endocrine disturbances such as gout, Graves' disease, etc., have more than the average number of female offspring. Bald men were found to have 40% more male offspring than men with full hair or with receding hairline that had not developed into full baldness. Male sex hormones play a role in the development of baldness.

Has the degree of maleness of the father an effect on the sex ratio of the children? We believe that men engaged in aggressive, extrovert occupations, in which few or no women have become outstanding, are more masculine than men engaged in introvert, retiring occupations. In families where the fathers are members of the armed forces, business executives, politicians, lawyers, farmers, abstract scientists like astronomers, mathematicians, etc., the sex ratio of 5,400 children was found to be 120 boys for every 100 girls. However, in families where the fathers were in professions in which many famous women were engaged—i.e., actors, social workers, child educators, fiction writers, and all kinds of artists—the sex ratio for 1,800 children was found to be 85 boys for every 100 girls born. An intermediate group was formed by the families in which the father was engaged in a religious profession, was a research worker, or an applied scientist such as a chemist, biologist, etc.

The author believes that the genes controlling the sex ratio in mammals are identical with, or act through, the genes controlling the male-female sex hormone balance. The X-bearing sperms, because of their ♀ chromosomal balance (1A:1S), form a foreign entity in the male reproductive organs, and are destroyed in smaller or larger number inside the male, depending

¹ Fulbright fellow.

on the degree of maleness. On the other hand, male fetuses form an alien factor in the maternal organism, and are thus more easily absorbed than female fetuses. This theory is in agreement with the one advanced by Hoelzel at the University of Chicago that in well-nourished male rats more X-chromosome-bearing sperms than Y-chromosome-bearing sperms are reabsorbed, whereas in well-nourished female rats more male than female fetuses are absorbed.

A Method for the Rapid Preparation of Histological Sections

John A. Tornabene and Edwin J. de Beer

*The Wellcome Research Laboratories,
Tuckahoe, New York*

The preparation of tissue specimens for paraffin embedding is a tedious and time-consuming process. The method described here considerably shortens the time required and reduces the number of operations. Particular economies in this respect have been achieved in the dehydrating step, which has been reduced to a single, simple operation.

Thin pieces of fresh tissue about 4 mm thick, are placed in a modified Bouin's fluid consisting of: 80% ethyl alcohol, 150 ml; 40% formalin, 60 ml; glacial acetic acid, 15 ml; and picric acid crystals, 1 g. A minimum of 35-45 min immersion is required to fix the tissues. No harm is done by permitting them to remain in the fixative overnight. Washing in water is not necessary. Zenker's fluid, Helley's fluid, etc., also may be used, provided that the proper prescribed procedure, including washing, is followed for each fixative.

After fixing in the modified Bouin's fluid, the tissues are cut into slices 1-2 mm thick. This can be done with a razor blade or sharp-edged knife.

Dehydration is rapidly accomplished by placing 4-8 slices of the fixed tissue in an Allihn filter tube (porosity of disk "medium," height above disk about 110 mm, capacity about 45 ml). Excess fixative is removed by rinsing with 5 ml acetone and decanting. The filter is then filled with pure acetone, which is allowed to run freely by gravity. When the tube is about half full it is refilled to the top with additional acetone. This process is repeated once again. By the end of an hour the filter will have emptied and the dehydration process will have been completed.

As soon as the last of the acetone has disappeared through the disk, the tissues are cleared with xylene. This may be carried out conveniently by pouring xylene into the Allihn tube. When the tissues become translucent they are removed immediately, since too long an exposure to xylene will render them brittle. Kidney slices may become translucent in 15 min, whereas spleen slices may require 45 min.

The cleared slices are placed in small, labeled, galvanized screen baskets, 1 in. in diameter and 3 in. high. These are placed in 250-ml beakers containing

melted paraffin (Tissuemat, mp, 54°-56° C) and maintained in a vacuum oven at 58° C and 560 mm Hg pressure. A desiccator, in an ordinary thermostatically controlled oven, connected to a Cartesian manostat with a vacuum filter pump, provides a satisfactory vacuum oven. After 30 min in the vacuum oven, the tissues are placed in a fresh beaker of pure paraffin and kept at 58° C for 30 min.

After the infiltration is completed, the slices are embedded in paraffin in the usual manner. It has been found useful on occasion to embed many tissues in the same block, using a cardboard box as a mold. If the box is large (3"×3" or larger) care must be taken to use flexible cardboard sides to permit the paraffin to contract on cooling without splitting the block.

In the method described, dehydration is first favored by the presence of 80% alcohol in the fixing solution. It is greatly intensified by the technique which makes use of the sintered glass filters. This procedure tends to promote a high concentration gradient between the water in the tissue and the acetone outside by constantly allowing the partly diluted acetone to escape through the bottom of the filter while simultaneously replacing it with fresh fluid from above.

The use of a partial vacuum helps to remove the xylene and thus to favor the infiltration by a paraffin that is comparatively pure. Too high a vacuum tends to separate and disrupt the tissues.

The method has been used routinely in our laboratory for more than a year. By its use, it is possible to start with fresh tissues in the morning and to complete the preparation of sections for microscopic study before the end of the working day. Good results have been obtained with such difficult preparations as those showing clearly the cilia of the respiratory epithelium or the ciliated brush borders of the proximal convoluted tubules of the kidney.

An Apparatus for Determining Bone Density by Means of Radioactive Strontium (Sr^{90})¹

F. Gaynor Evans, Carl C. Coolbaugh,
and Milton Lebow

*Departments of Anatomy and Engineering Mechanics,
Wayne University, Detroit, Michigan*

During investigations upon regional differences in the physical properties of the compacta of the leg bones of man and the dog the density was one of the properties studied. Since one of the investigators (CCC) is studying the effects, in the dog, of alterations in the blood supply of the femur, it was necessary to have a method for detecting very slight differences in density. It was therefore decided to determine the density by the percentage transmission of β -rays through the bone samples.

¹ This investigation was supported (in part) by a research grant from the National Institutes of Health, USPHS.