ribonuclease activity. Similar changes in RNA and ribonuclease were noted in aging apple leaves(9).

When tumor-bearing rats were treated with PEDP there was a marked reduction in size of the tumor as compared to non-treated controls (Fig. 2). The smaller tumors in the animals treated with drugs had a higher ribonuclease activity and a lower RNA-P content than the controls. The DNA-P per mg of tissue was also decreased in treated tumors which suggested a smaller number of cells per gram of tumor tissue. If ribonuclease activity was calculated relative to DNA-P concentration, the values were: $29.5 \pm 1.3 \times 10^2$ and $6.05 \pm 0.39 \times 10^2$ units per mg DNA-P, respectively, for treated and non-treated tumors. Similarly, the RNA/DNA ratio for DEPA-treated tumors was 0.37 ± 0.04 while the non-treated 0.74 ± 0.06. Slower growing tumors, therefore, had an increased ribonuclease activity which also could be correlated with a low RNA concentration per cell.

Protein concentration per mg DNA-P was increased from 150 ± 12 to 224 ± 45 in PEDP-treated tumors. Previous work(10) has demonstrated a marked effect of ethylenephosphoramides upon cell nuclei, producing bizarre, polymorphic, giant cells with distorted nuclear and cytoplasmic configuration. These facts might explain the increased protein/DNA ratio and decreased DNA concentration found in these tumors.

Summary. As the Walker 256 tumor increased in size and approached maximum development, the ribonuclease activity per gram of tumor or per mg of deoxyribonucleic acid phosphorus (DNA-P) also increased. The slower growing, larger tumor had a markedly decreased ribonucleic acid phosphorus (RNA-P) and protein concentration. Treating the tumor-bearing rat with N, N′-diethylene-N″-phenethylphosphoramide (PEDP) resulted in reduced rate of growth of the tumor, increased ribonuclease activity, and decreased RNA-P.

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Preparation of Chromosomes for Light Microscopy Utilizing an Agar Fixation Procedure. (27899)

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Determination of chromosome morphology and number is becoming increasingly important in both clinical practice and as a research aid. Some laboratories routinely monitor their cell cultures and follow the chromosome number as a check on the homogeneity of the cell population. Determination of chromosome number and morphology also provides a means for study of chromosomal change and stability under the action of various chemical and physical agents. In order that such studies can be carried out efficiently, a rapid precise means is needed for monitoring the chromosomal constitution of cell lines.

Many methods are now available for preparing chromosomes for examination; however these are quite time consuming(1,2,3).

This note describes a simple procedure for rapid preparation of mammalian cells for delineation of chromosomes. The method employs a modified agar fixation technic similar to that introduced for cytological and morphological studies on microorganisms(4,5,6,7). In this study the technic has been applied to several lines of mammalian cells cultivated *in vitro* and to an ascites tumor of mice.

Materials and methods. Cells. HeLa, mouse tissue strain "L" and bovine kidney (8) were used in this study. Standard procedures were used for maintenance of cell stocks. The ascites tumor was Sarcoma I grown in the peritoneum of A/Jax strain of mice.

Preparation of cells for expansion. Cell monolayers, grown in the absence of colchicine, were removed from the glass with 0.05% trypsin or with a mixture of 0.05% trypsin and 0.02% EDTA (3:1). The cells were then centrifuged at 800 rpm for 5 to 10 minutes, the supernatant decanted and the cells resuspended in growth medium (10% calf serum and 0.1% yeast extract in Hanks' Balanced Salt Solution), to a final concentration of 2×10^6 to 4×10^6 cells per milliliter. The ascites tumor was withdrawn from the peritoneal cavity of a mouse and diluted with growth medium to the above cell concentrations.

Expansion of cells. An agar surface was used to expand the cells and disperse the chromosomes. The agar medium consisted of distilled water containing 1.5% Difco agar and 0.5% sucrose. Twenty to twenty-five ml of the agar was poured into a 15 × 100 mm petri dish and allowed to harden. The plates were then inverted and dried overnight at 37°C.

One-hundredth ml of the cell suspension was spotted on the surface of the agar and allowed to stand until excess moisture was absorbed, at which time the spot had a dull appearance. This absorption required 10 to 15 minutes.

Fixation. An agar square slightly larger than the spot was cut and removed from the plate and placed cells down on a coverglass, which was then immersed in fixative. When the cells became fixed, the agar block was rapidly lifted from the coverslip leaving a thin film of cells attached to the glass. The coverglass was stored in 70% alcohol prior to staining.

In this investigation both Bouin's and Carnoy fixatives were employed. The fixation times were 30 minutes and 25 minutes respectively for Bouin's and Carnoy fixatives.

Staining. The cells were stained with Giemsa stain or by the Feulgen procedure with basic fuchsin.

Results and discussion. Using the technic described above many preparations were made of each cell type investigated. Each of these preparations showed numerous cells suitable for the study of chromosome number and morphology. Figures 1, 2 and 3 represent selected examples of HeLa, ascites tumor, and bovine kidney cells prepared by this method. In these cells the number of chromosomes can be determined easily as can the relative length of each chromosome and the position of the centromere. It should be pointed out that the cytoplasmic membrane appears to be relatively intact in most of the cells. Although this is not evident in figures 1, 2 and 3, it is much more apparent in non-hydrolyzed preparations stained with Giemsa stain or by hematoxylin and eosin.

The procedure has further merit in that it is applicable to uncultured blood cells and ascites cells or to cultured cells that will not adhere to glass. It can also be used for cell suspensions which normally are fastened to glass for other staining procedures.

During the course of this investigation it was found that sucrose concentrations above 2% progressively inhibited cell expansion. Figure 4 shows a HeLa cell in metaphase prepared on agar containing 8% sucrose. Spindle fibers were readily observed microscopically although they are only faintly visible in the photograph. Cells in anaphase and telophase were also observed at these increased concentrations of sucrose. This suggests that the technic reported here could be used for the study of cells in all stages of mitosis.

From preliminary work, it appears that this technic can be applied to blood cells or cell suspensions from trypsinized tissue.

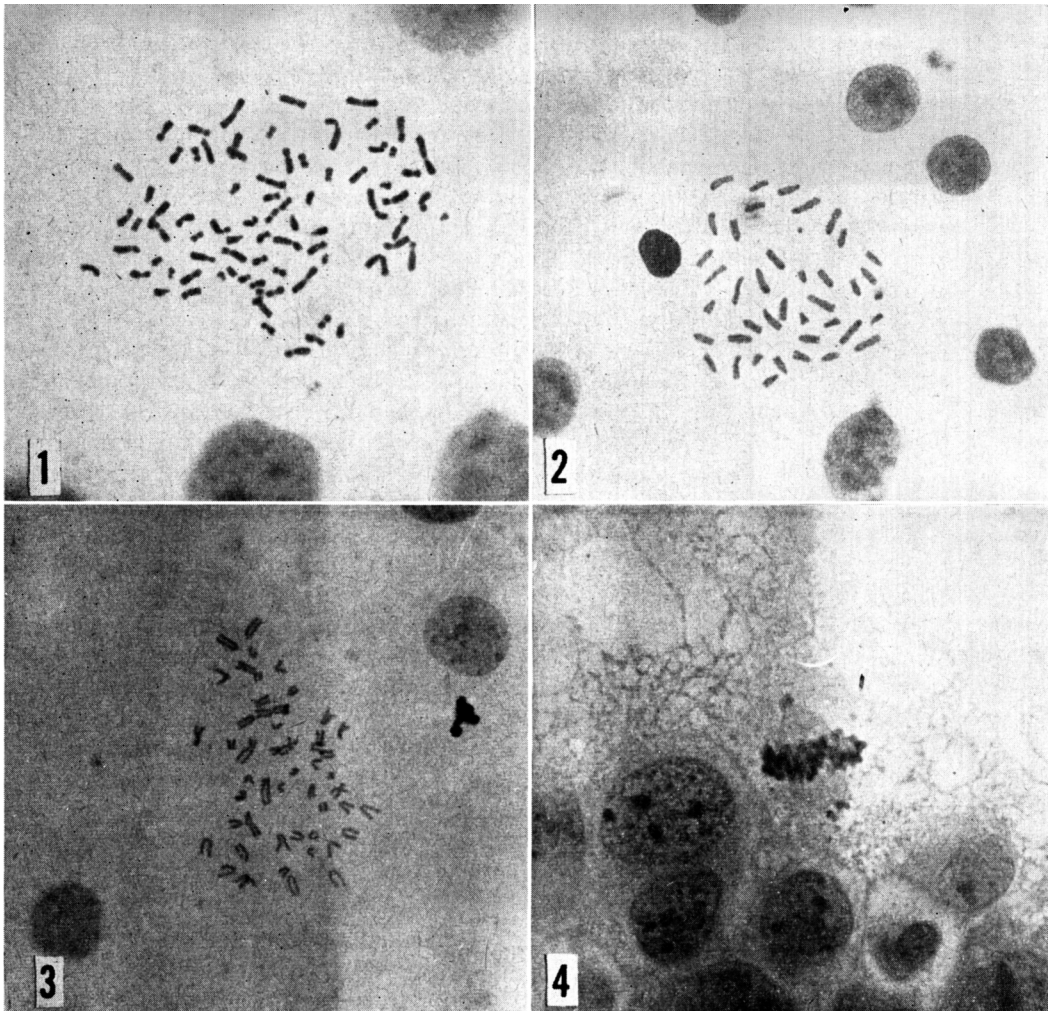

FIG. 1. HeLa cell stained with Giemsa stain. (X1100)
FIG. 2. Ascites tumor cell stained with Giemsa stain. (X1200)
FIG. 3. Bovine kidney cell stained by the Feulgen procedure. (X1100)
FIG. 4. HeLa cell in metaphase showing spindle fibers. Stained with hematoxyln and eosin. (X1100)

Summary. A rapid reliable agar fixation method for delineation of chromosomes has been applied to suspensions of cultured and uncultured mammalian cells. Various stages of mitosis have been observed in these cells by controlling the osmotic strength of the agar. The technic is also of value for preparing cell suspensions for routine staining procedures.

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