only a few contaminating blood and mesothelial cells. In Fig. 4 photomicrographs from the same preparations are shown at half the magnification. A comparison of Fig. 4 with 4a reveals the amount of concentration of malignant cells which has taken place. In the control smear, Fig. 4, three cancer cells can be made out, while in the same area of Fig. 4a, prepared after flotation, a score or more can be identified.

This study has revealed information about the specific density of certain malignant cells found in pleural and abdominal fluids of cancer patients, making possible an improvement in the present method of examining such fluids for the diagnosis of cancer. Apart from its possible clinical value, the method shows promise of providing means of obtaining relatively pure populations of neoplastic cells for chemical and physical investigations. Experiments now in progress suggest that, with certain modifications, the method may be applied to the concentration and segregation of desquamated malignant cells in vaginal fluids by elimination of certain other cell types from the total population.

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A Multiple-Standard, Vertical-View Comparator for Microdeterminations

Henry O. Schulze

Bethesda, Maryland

The author developed the apparatus to be described in response to needs of the Department of Bacteriology of the Johns Hopkins University School of Medicine. When used for pH determinations, this device, based on the same colorimetric principle as that of the horizontal-view comparator of Gillespie (1920), employs three glass cups arranged in a vertical series to form a standard against which is compared a single test cup containing a specimen sample with 1 ml of indicator solution. Light passes upward from the light-source base through the cups to the observer. Fig. 1, a diagrammatic cross section through the comparator, summarizes the principle of the cup and comparator design in a working arrangement using Gillespie "drop-ratio" standards.

The light-source base, a box, the inside surfaces of which are painted dull white, has a sloping frosted glass window across the entire back side (not shown in Figs. 2 and 3) and a sloping front side. This design serves better to receive the light of the usually available illumination and distribute it uniformly over the reflecting floor of the base. A sliding panel may be grooved into the floor so that painted standards may be used for rough work.

The base supports two trays, "cup racks," one above the other. These may be seen in the unassembled view, Fig. 2. The lower rack has been left on the base. Dowels

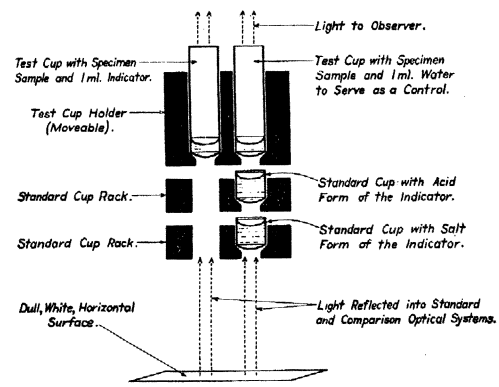

FIG. 1.

keep the racks in proper alignment. In the comparator shown provision has been made for the simultaneous use of two indicators, that is, each rack has two rows of "cup holes" separated by a middle row of simple "optical holes." Each row has thirteen holes and the cup racks

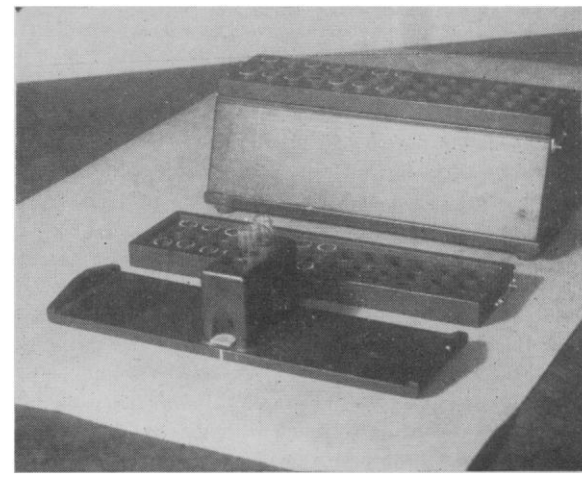

FIG. 2.

are identical with respect to their plan views. In Fig. 2 some cups are left out of the cup holes of the front and back standard row better to expose these holes to view. Metal hooks are attached at the ends of the base and these engage pins in the upper cup rack, thereby serving to hold the cup racks and base together as a single unit.

Finally, there is the "test cup holder" which slides on top of the upper cup rack. This has three cup holes. A test cup containing indicator and specimen may be placed in the middle hole and conveniently compared with standards of the same indicator by moving the holder into positions over the various standards in the cup racks below. Just below the test cups there is a slot in the block of the test cup holder into which color filters[1] may be inserted. All surfaces, except the inside of the base, are painted dull black.

[1] Wratten filters, obtainable at Eastman Kodak stores, are satisfactory.

All the cup holes are $\frac{11}{16}$ in. in diam and are spaced $\frac{1}{4}$ in. apart in both directions. At the bottom of every cup hole is an inward flange leaving an optical hole $\frac{1}{2}$ in. in diam. These flanges serve to support the cups and to restrict the light paths to the central areas of the same. A last important dimension is the distance between the floor and the ceiling of the light-source base. This should be not less than $3\frac{1}{2}$ in.

The cups used with this comparator are made from flat-bottomed glass shell vials, 17.0 mm outside diam and 75 mm long. The flat bottoms are softened in an air-blast flame and blown out 1.5 to 2.5 mm beyond the bottom edges of the vials. The resulting convex curvature is more than sufficient to compensate for the concavity of the central areas of the meniscuses of the aqueous solutions contained in the cups. This operation results in test cups. Standard cups are made by cutting off test cups to a length of 13 mm. The test cups used to make standard cups must be selected so that the inside diam may not vary more than 0.2 mm if Gillespie standards are to be used. If buffer standards are to be used, the variation may be 1.0 mm. Vials which have entirely flat and uniform bottoms may be used without blowing out for test cups or standard cups, but all solutions placed in such cups must be layered with a clear white mineral oil or mineral spirit so that flat meniscuses are formed.

Accessory equipment and materials are as follows: (1) A mixing pipette which has a small delivery opening and is fitted with a 2-ml rubber bulb. (2) Pipettes, Mohr type, 1-ml capacity and 0.01-ml divisions, not graduated to the tip. (3) Distilled water in a suitable Pyrex or other chemically inert bottle or flask. (4) A solution of $N/1$ NaOH in a bottle with a dropping pipette. (5) A solution of $N/1$ HCl and (6) a solution of 1% acetic acid in dropping bottles. (7) Solutions of indicators in 20% alcohol and adjusted to near their respective pK values. The indicators are used in concentration of 0.004% or 0.006%.

Procedure for preparing the standards is as follows. The cup racks are placed before the operator as shown in Fig. 2. The cup holes are filled with clean dry cups. One-ml amounts of the desired indicator solution are divided between vertical pairs of cups in a standard row according to the drop-ratio system of Gillespie. Begin with the first pair of cups at the left end, adding nothing to the upper cup and 1.0 ml indicator solution to the lower. To the next pair add 0.1 ml to the upper cup and 0.9 ml to the lower cup. Complete the distribution of drop-ratio aliquot amounts to the other pairs of cups from $1\frac{1}{2}$: $8\frac{1}{2}$ to 9: 1. The reader is referred to the chart by Gillespie giving pH values for the drop ratios for various indicators. To the last pair of cups at the right there is added to the upper one the entire 1.0 ml of indicator solution. If the use of a second indicator is desired, a set of standards is prepared in the same way at this time in the other standard row. To every cup in the upper cup rack is added one drop of alkali solution and to those in the lower rack, one drop of acid solution. Then, with the mixing pipette, a brisk jet of distilled water is added to each cup so that the rims of the meniscuses are about 1 mm below the rims of the cups. The cup racks are then replaced onto the base and made fast with the hooks. On this assembly the test cup holder is placed. The comparator is placed so that the window is squarely facing a light source and is then ready for use (Fig. 3).

Exactly 1.0 ml of the indicator solutions used for making the standards is pipetted into the test cups. Approximately 1.0 ml of distilled water is added to the other (control) test cups. The cup containing indicator is placed in the central hole of the test cup holder and one containing water, over the standard to be used (see Figs. 1 and 3). The specimen (broth culture or solution) to be tested is added drop wise, alternately, to both cups until there is no longer any change of color in the cup containing indicator. The color of the specimen and indicator mixture is then compared with the standards by moving the test cup holder along until the closest match is obtained. The drop-ratio pH chart is then referred to for assigning a pH value to the specimen.

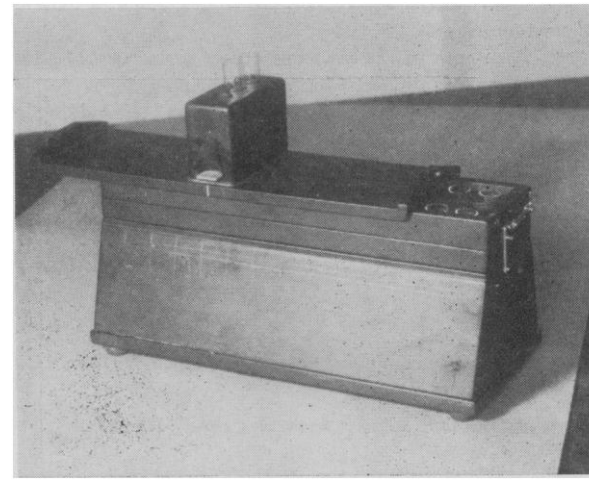

FIG. 3.

The exploratory stage of making determinations may be dispensed with after one is confident of the minimum amount of specimen to use. This is true particularly of bacteriological media and cultures. Practically the same amount is necessary for making determinations, regardless of the pH, in the case of a given medium that is normally within the pH range of such solutions. During the exploratory stage the operator will become aware of the buffer effect of indicator solutions. This effect is minimized, but not eliminated, by having the indicator solutions adjusted to near their respective pK values (their intermediate tint) and at as low concentration as can be satisfactorily used.

The choice of color filters is left to the operator. Tizard (7) obtained better results with the use of the yellow light of a carbon filament lamp while measuring color densities of methyl orange and of methyl red at various hydrogen ion concentrations. Clark and Lubs (3) recommend the use of a light screen made of translucent paper coated with an acid solution of phenol-sul-

fonphthalein to eliminate dichromatism as encountered with bromcresol purple.

The advantages of this comparator are as follows:

1. It minimizes eyestrain, the vertical view arrangement resulting in (a) a flat uniform field of light and (b) the elimination of direct glare from the light source which is often experienced with horizontal view devices, and the reflected glare from white opaque plates used in other microtechniques.

2. Good results may be obtained with twilight that is yet strong enough to read newsprint.

3. By the inclusion of maximum-minimum control standards and the use of partial color filters the entire range of an indicator may be used with good results.

4. It is adaptable to the aseptic technique used by bacteriologists. This minimizes the danger of splattering culture over the field of operation, such as is a hazard with the methods of Brown (*2*) and Felton (*4*).

5. The test cups are disposable in the same manner as are culture tubes, eliminating the hazard of infection from improperly disinfected electrodes when a potentiometer is used.

6. The depth of the test cups allows room for (a) adding enough of a poorly buffered specimen to overcome the buffer effect of the indicator solution, (b) adding a deep layer of oil to protect the test from the atmosphere if so desired, and (c) titration of 1.0 ml or less of a specimen for determination of total acidity or the buffer index (*1*).

7. The accuracy is comparable with that of the Gillespie method (*5*) under optimum conditions.

8. There is provision for a specimen control cup which is not employed in the methods of Brown (*2*), Felton (*4*), and Haas (*6*).

9. Convenience and rapidity of operation result from the mechanical design and from the constant indicator intensity, regardless of the depths of the solutions in the test and standard cups. The apparatus is much simpler to use than to describe.

10. The device is adaptable to other types of colorimetric and turbidometric determinations.

11. The use of reflected light makes possible the use of permanent painted standards.

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Effect of Panparnit on Brain Wave Changes Induced by Diisopropyl Fluorophosphate (DFP)

C. F. Essig, J. L. Hampson, P. D. Bales, Alice Willis, and H. E. Himwich

Medical Division, Army Chemical Center, Maryland

The intracarotid injection of the anticholinesterase, diisopropyl fluorophosphate (DFP) in curarized rabbits produces grand mal-like electroencephalographic (EEG) patterns (*2*). These abnormal brain waves are associated with extremely low cholinesterase levels of cerebral cortex, midbrain, cerebellum, and medulla (*4*). We are thus confronted with presumptive evidence that DFP acts as a convulsive agent by permitting the accumulation of acetylcholine. This proposed mechanism receives some support by virtue of the ability of atropine to prevent or abolish the grand mal-like EEG patterns (*2*). However, trimethadione (tridione), sodium pentothal, and phenobarbital, although not anticholinergic drugs, have a similar effect on cerebral convulsive activity induced by DFP (*6*). It is interesting to note that none of these anticonvulsant agents raises the depressed cholinesterase levels in the brain (*6*).

Panparnit, a drug with an imposing and diverse array of pharmacologic actions, has been added to the list of drugs reportedly effectual in treating postencephalitic Parkinsonism (*3*). This drug (diethylaminoethylester of phenylcyclopentane carboxylic acid) is grouped with the synthetic smooth muscle spasmolytics. Panparnit further resembles atropine in producing relatively mild anticholinergic effects on the vegetative nervous system. Domenjoz, who described these Panparnit characteristics, also states that it has a curariform effect on frog skeletal muscle (*1*). Gruber and associates (*3*) report that this agent abolishes decerebrate rigidity in cats by virtue of a central mechanism. Heymans and Estable (*5*) describe Panparnit as being anticonvulsant. These authors also state that Panparnit protects against high doses of acetylcholine, pilocarpine, diisopropyl fluorophosphate (DFP), strychnine, and metrazol. It was decided to test, by electroencephalographic assay, whether the anticonvulsant property and the protective function of Panparnit would combine to make this drug an effective curative for DFP-induced grand mal-like convulsions.

Albino rabbits were prepared under local procaine anesthesia (2%). The trachea was cannulated for artificial respiration, both carotid arteries were exposed for the injection of DFP (1 mg/ml in distilled water), and steel electrodes were pressed through the skull over each cerebral hemisphere. Monopolar corticograms from each side of the brain and an electrocardiogram were recorded simultaneously on a four-channel Grass apparatus. The animals were curarized and placed under artificial respiration. Small doses of atropine (0.02 mg/kg) were used, sufficient to protect the heart until grand mal-type brain waves were obtained. This dose of atropine is too small to abolish cerebral hyperactivity. DFP was injected into one carotid artery at 12-min intervals in doses of 0.5 mg/kg, a little higher than the LD_{50}. Two to three such doses were required to produce bilateral grand mal-like