adapt photoelectric methods to the direct counting of microscopical cells in suspension in water.

A capillary glass tube, made from a small tube elongated over a gas flame, is placed under the high magnification field of a microscope. The microscopical cells in suspension in water (red blood cells or neutral-red stained yeast cells) are forced under pressure to circulate through the capillary tube. A photoelectric apparatus of the smaller type is adjusted to the microscope's ocular and connected with an appropriate meter. The purpose of the experiment is to have each microscopical cell passing through the capillary tube, register itself automatically on the photoelectric apparatus, thus creating a micro-current which can be amplified and recorded.

The technical difficulties to overcome in such an experiment can be listed as follows:

(1) Difficulty to standardize capillary tubes in such way as to fill exactly the highest magnification field.

(2) Difficulty to flatten capillary tubes (as suggested by Sturges in his studies on bacterial motility) to insure proper focus.

(3) Necessity to shake dilution samples thoroughly to prevent elumping of cells in capillary tubes.

(4) Desirability of a specific photoelectric apparatus highly sensitive to microscopical objects. The ordinary commercial photoelectric apparatus is not built or intended for such purpose and shows only a faint reaction to magnified erythrocytes, neutral-red stained yeast cells or microscopical solid particles.

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## A NEW SOURCE OF ILLUMINATION ADAPTED TO PHOTOGRAPHY AND LOW-POWER MICROSCOPY

An adaptation of the so-called neon type of tubular light has given promise of furnishing satisfactory illumination both for the photography of small opaque objects and for the binocular microscope stage. The apparatus is made in the usual arrangement used for the neon type, namely, glass tubing filled with a combination of gases, which may be varied to control the spectrum range. A step-up transformer on the 110-volt lighting circuit supplies a potential of 2,000 or more volts across electrodes fused into each end of the tube. For the purposes mentioned the glass tube was shaped into a number of close, circular turns, forming either a single or double spiral. For use with the microscope a spiral of three turns has been found satisfactory, but for photography a larger number is more desirable to furnish as much intensity as possible. When in use the lighting unit is placed around the binocular microscope objective, about two inches from the object, or around the camera lens so that the photograph is made through the opening in the center of the unit. The inside diameter of the unit should be not less than two inches. A sleeve extending inside the light and fitted to a reflector back of the light shades the objective or camera lens, as well as increasing the efficiency of the apparatus. An arrangement to cover a variable sector of the unit allows control of the intensity and angle of the shadows so that perspective may not be lost.

This type of lighting is particularly advantageous because of the absence of intense heat, which would be detrimental to lens mountings or to the objects. The intensity of the light source is very even over all its area, so that even lighting of the object is obtained with no manipulation, and it is very easy to control the shadow intensity to suit the subject. Also there is no flare, such as is found in some forms of incandescent lighting. The cost of this type of unit is under ten dollars.

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### LANTERN SLIDES ON "FROSTED" GELATINE

SEVERAL communications relative to the use of Cellophane as a vehicle in the making of lantern slides have appeared recently in SCIENCE. With our aluminum-surfaced screens we find an undesirable glare develops even when yellow-tinted Cellophane is used. Furthermore, unless great care is taken, Cellophane does not take ink uniformly and exactness in delineation is sometimes sacrificed by this effect. Where compass work is required, however, Cellophane is far more convenient than commercial slides of clear or "etched" glass.

In searching for substitutes for Cellophane which will eliminate the difficulties mentioned we have found that *thin*, translucent, onion-skin paper is excellent. Colored inks provide for differentiation in diagrams when desired.

Better than the paper is a "frosted"—the trade term—gelatine sheet for flood lights by Klieg. When ink is applied to the matte surface of this sheet the roughening particles appear to dissolve, coalescing into a smooth, transparent surface when dry. Lines then appear in color on a gray background when projected. A startling fact is that perfectly white lines appear on the gray surface when drawn with plain water. With ink or water the change to transparency is immediate, drying almost as rapid, so that the slide works up very quickly. Details may be worked in with lead of pencils when required.

We have found that the gelatine sheets offer only one disadvantage—they are so sensitive to contact with water that impressions of the worker's fingers are recorded if the latter are damp. Wiping the fingers with alcohol, xylene or other "dryers" is helpful in avoiding this trouble.

By this method the cost of a slide is not more than

## AUTOCATALYTIC ACTIVATION OF TRYP-SINOGEN IN THE PRESENCE OF CON-CENTRATED AMMONIUM OR MAGNESIUM SULFATE

THE writers have described<sup>1</sup> the isolation from fresh cattle pancreas of a crystalline protein ("chymo-trypsinogen") which is transformed by a minute amount of active trypsin into an active proteolytic enzyme "chymo-trypsin." The course of the activation reaction is monomolecular and its rate is proportional to the trypsin concentration. Chymo-trypsinogen can not be activated by entero-kinase while the mother liquor from the chymo-trypsinogen crystallization is activated by entero-kinase but not by trypsin under ordinary conditions.

Subsequent experiments have shown that a protein fraction which has a very slight activity can be obtained from this inactive mother liquor. This fraction becomes highly active, as measured by the digestion of hemoglobin or casein, if allowed to stand for several hours in the form of a suspension in 0.5 saturated ammonium or magnesium sulfate at about pH 7.0 and 30° C. The activation follows the course of an autocatalytic reaction except for a prolonged lag period. The final specific activity is about 80 per cent. of that of crystalline trypsin. If a fresh suspension is inoculated with some of a perviously activated suspension activation occurs very rapidly. Active trypsin may thus be "propagated" by inoculating a suspension of the inactive protein with active material.

The suspension is prepared for activation as follows. The mother-liquor from the chymo-trypsinogen crystallization previously described is precipitated by bringing to 0.7 saturated ammonium sulfate and filtered. One gram of this filter cake is dissolved in 7.5 ml M/5 phosphate or borate buffer pH 8.0 and then 7.5 ml saturated ammonium sulfate is added. The suspension contains about 1.5 mg of protein nitrogen per ml.

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1 M. Kunitz and J. H. Northrop, SCIENCE, 78: 558, 1933; Jour. Gen. Physiol. (in press).

one third that of an etched slide of similar appearance prepared with transparent "inks."

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# SPECIAL ARTICLES

#### CRYSTALLINE PROGESTIN

The preparation from corpus luteum extract of crystalline material possessing progestin activity has been reported by Fels and Slotta,<sup>1</sup> Fevold and Hisaw,<sup>2</sup> and Allen.<sup>3</sup> None of these workers gives details as to the physical and ehemical properties of their preparations. In a joint investigation carried on in the Rochester and Columbia laboratories we have succeeded in isolating several crystalline compounds from the product obtained by Allen's procedure. The main constituent of the mixture is a physiologically inactive compound, A, melting at 190° and possessing the composition  $C_{21}H_{34}O_2$ . This compound is a hydroxy ketone; its phenylurethane, p-nitrobenzoate and semicarbazone have been prepared.

A compound, B, with the formula  $C_{21}H_{30}O_2$  crystallizing from ether-petroleum ether in blunt prisms with a melting point of 128° proved to possess the characteristic physiological properties of the hormone. It causes progestational proliferation in the uterus of the castrated rabbit in doses from 0.5 to 1.0 mg. A potency of 1 rabbit unit per mg has been tentatively assigned to this compound. Since the compound yields a crystalline dioxime, both oxygen atoms must be present in the form of carbonyl groups. The ultra-violet absorption spectrum of this compound shows a single band with a maximum at 240 mµ, which according to Menschick, Page and Bossert<sup>4</sup> is characteristic for  $\alpha,\beta$ -unsaturated ketones. Compound A in the same concentration does not absorb light in the photographic region.

Furthermore, a compound, C, melting at 120–121° and crystallizing from ether-petroleum ether or dilute methyl alcohol in needles, has been isolated. This substance is also physiologically active; its potency is the same as that of Compound B within the limits of accuracy of the assay. Its ultra-violet spectrum is identical with that of Compound B. On combustion it gives the same figures for hydrogen as B, but somewhat lower carbon figures. On treatment with semicarbazide both compounds C and B yield apparently the same amorphous semicarbazone, which is

<sup>1</sup> E. Fels and K. H. Slotta, Klin. Woch., 10: 1639, 1931.

<sup>2</sup> H. L. Fevold and F. L. Hisaw, Proc. Soc. Exp. Biol. Med., 29: 620, 1932.

<sup>8</sup> W. M. Allen, Jour. Biol. Chem., 98: 591, 1932.

<sup>4</sup> W. Menschick, J. H. Page and K. Bossert, Ann. Chem., 295: 225, 1932.