we demonstrated the ability of epicaine to produce definite sensory anesthesia within one minute in a 1 per cent. strength. We further studied the cat's blood pressure, the uteri of the guinea pig and cat, the gut of the cat, rabbit and monkey, the excised frog's eve and the pupil in the intact cat. In all these the effect was sympatheticomimetic in type.

A more complete study of the pharmacological actions of this drug is now in progress and will be reported in the near future. RAYMOND L. OSBORNE

## SCIENTIFIC APPARATUS AND LABORATORY METHODS

## DIFFERENTIAL STAINING FOR LIVING AND DEAD CELLS

In a series of investigations on the mechanism of cellular death with the epidermal cells of Allium cepa, I used as a method of differential diagnosis of living and dead cells either the classical Ruzicka's<sup>1</sup> stain (methylen blue and neutral red) or some staining mixtures proposed by later investigators, as, for example, Becquerel's<sup>2</sup> stain (methylen blue, neutral red and Bismarck brown). With these stains the difference in color between the living and the dead cells is often sufficient for a reliable diagnosis of death, but it is never very "contrasty," and most of the time one wishes for a more definite criterion. In search of sharper contrast I tried the simple method of first using a stain which would penetrate both the living and the dead cells and afterwards of applying a reagent which would enter the dead cells only and modify in them alone the color of the stain. The results were, as far as contrasts are concerned, beyond all expectation.

The procedure is as follows: A piece of the lower epidermis of the scale of the onion bulb is peeled off and placed, cutin side down, on a slide. A drop of a .5 per cent., slightly alkaline, aqueous solution of neutral red is deposited on the piece of epidermis and left there for 2 minutes; then it is blotted off and replaced by a drop of a .4 per cent. potassium hydroxide solution, which is immediately removed (also with a blotter); then the preparation is washed with tap water. The living cells take with that treatment a bright cerise red color, while the dead cells are of an intense orange yellow. The contrasts are violent. There are intermediate tints which correspond to the dying cells.

A cell treated with a .4 per cent. solution of KOH may stay alive for hours (if the piece of epidermis is to be observed without cover-slip, put its cutin side up, to avoid evaporation). Lower concentrations of KOH can be used, but they should be applied for a longer time; higher concentrations up to 1 per cent. were also used successfully; above 1 per cent. they become too injurious, but they are not instantly lethal up to 2.5 per cent.

<sup>1</sup> V. Ruzicka, Pflügers Arch., 107: 473, 1905.

<sup>2</sup> P. Becquerel, Comptes Rendus Ac. Sc. 176: 601, 1923.

With solutions of neutral red weaker than .5 per cent., the staining process requires a longer time.

The correspondence between the vitality of the cells and their color has been ascertained by testing cells of a given tint for their ability to plasmolyze, their ultra-violet (Luyet and absorption Gehenio's<sup>3</sup> method), and their permeability to various vital and non-vital stains.

Paradoxical as it may seem, the living cells become redder when the action of KOH is prolonged. This is probably due to the production of an "acid of injury" within the cell.

The method employed has been found successful particularly with cells which possess a well-developed vacuolar system. This is consistent with the fact that neutral red, as a vital stain, acts specifically on the vacuome (cf. Guillermond<sup>4</sup>).

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## THE PREPARATION OF FINE FILAMENTS

THE special article entitled "Simplified Preparation of Microscope Cross Hairs"<sup>1</sup> by A. Wilson Footer indicates a most important method for the easy production of fine filaments of desired size. The dissolving of the silver coating on fine (Wollaston) wires of 0.0001 inch in diameter or 0.00005 inch in diameter and the securing of the core wire intact is an operation with which only the skilled technician is likely to have success.

However, using the commercial adhesive (I understood this to be Duco Household Cement, 10-cent tube), it is possible after a few minutes' experimentation to draw out with the aid of a probe short filament sections, having diameters as small as 0.00005 inch, which can be easily mounted on grids.

Measurement of exact diameter of a section of the filament may be made with a filar micrometer.

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<sup>3</sup> B. J. Luyet and P. M. Gehenio, Biodynamica, No. 11, 1936.

4 A. Guillermond, "Les Constituants Morphologiques du Cytoplasme: Le Vacuome," p. 10. Paris, 1935. <sup>1</sup> SCIENCE, 84: 490, 1936.