cum × *N. glutinosa;* similar F_1 plants have been produced and tested several times in the past.[2] All plants of this first hybrid generation have proved sterile. Because of this sterility, transfer of the gene to further generations was accomplished only by the use of *N. digluta* (fertile amphidiploid *glutinosa-tabacum*). There was uniform necrotic-type response in all plants of the generation *N. digluta* × *N. tabacum.* Segregation occurred in the subsequent backcross generation (*N. digluta* × *N. tabacum*) × *N. tabacum,* mottling-type plants being present in excess of expectation.

It is not yet known whether these necrotic-type genes characteristic of *N. rustica* and *N. glutinosa* can be incorporated in strains of *N. tabacum.* The work here reported is being continued with a view to securing an answer to this problem, because it is believed that tobacco mosaic would be unable to maintain itself in tobacco varieties bearing these genes, and that this disease, now prevalent in such other crops as tomatoes and peppers, might disappear if the virus reservoir in tobacco were eliminated or considerably reduced.

F. O. HOLMES

THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
PRINCETON, N. J.

A VASOPRESSOR LOCAL ANESTHETIC[1]

ONE of the disadvantages of procaine (novocaine) and its known relatives is that they tend to dilate the peripheral vessels. They cause a pronounced fall in blood pressure[2,3,4] as does cocaine,[5] in the ordinary concentrations.

We report the discovery of a new local anesthetic which is vasopressor.

The action of cocaine is dependent upon the presence of a nitrogenous nucleus, an esterified carboxyl and a benzoyl group. The non-alkaloidal local anesthetics owe their action to the benzene nucleus, a carboxyl esterification, and the presence of an amino group para to the above carboxyl.

On these assumptions the search for ideal local anesthetics has proceeded in two directions: (1) derivatives containing a cyclic nitrogen, and (2) derivatives of the amino and oxyamino benzoic alkyl ester type.

Koller[6] first discovered the anesthetizing effect of cocaine on the tongue and in the eye in 1884. Einhorn[7] produced local anesthesia in 1899 by esters of aminobenzoic acid. He introduced procaine, better known as novocaine (p-aminobenzoylbetadiethylaminoethanol), in 1905.

In 1903, Braun reported that the anesthetic effect of cocaine is greatly enhanced by the addition of epinephrine,[8] which is similarly efficient with procaine. Procaine dilates the blood vessels and hastens the exit of the anesthetic from the site of action. With epinephrine the duration of anesthesia is prolonged for over an hour; thus the concentration of the anesthetic can be considerably lowered; the danger of poisoning is also decreased. These results are due to vasoconstriction produced by epinephrine, which practically arrests absorption into the circulation. On the other hand, the nervousness induced by epinephrine in hypersensitive individuals is objectionable; further, it may make them susceptible to cocaine collapse. The epinephrine combination is useless on the intact cornea and for intravenous and subdural injections.[9] The greatest use of the epinephrine combination is in operations involving bleeding; although cocaine is somewhat hemostatic, procaine and the other known synthetic derivatives tend to increase bleeding.

Thus epinephrine is used with all the local anesthetics of *both* types to prevent general absorption and combat the depressor effect of the anesthetic.

In 1931, under the supervision of Professor Nelson and Dr. Powell, of the Department of Chemistry of Columbia University, we began the preparation of a compound that would combine the actions of epinephrine and of procaine. After discarding many such drugs because of undesirable effects we have finally synthesized alpha (3, 4-dihydroxyphenyl) beta (para-aminobenzoylbetadiethylaminoethanol) alphaethanone-hydrochloride, designated for brevity as *epicaine,* which is both a local anesthetic and vasopressor.

We have shown that sympatheticomimicity is greatest in a compound in which the phenyl-hydroxyls are in the 3, 4-position, there is a two-carbon side-chain, a beta-carbon hydroxylated, and an alpha-carbon hydrogen substituted by some indifferent molecule, preferably an amine (but not necessarily so), and, where all these are present, a levorotatory isomer.[10] Our new compound contains these as well as the requisites enumerated above for local anesthesia.

By Rider's method[11] using the frog's sciatic plexus

[2] H. A. Allard, *U. S. Dept. Agr. Bull.,* 40, 1914; J. Johnson, *Am. Jour. Bot.,* 23: 40, 1936; F. O. Holmes, *Phytopath.,* 24: 984, 1934.

[1] From the DeLamar Institute of Public Health, College of Physicians and Surgeons, Columbia University.

[2] Roth, *Hyg. Lab. Bull.,* 109, 1917.

[3] Roth, *Jour. Pharm. and Exp. Ther.,* 9: 352, 1917.

[4] Osborne (to be published).

[5] v. Anrep, *Arch. ges. Physiol.,* 21: 38, 1880.

[6] Koller, *Sitb. Wien. Akad., Math. N. W. Kl.,* Nov., 1884.

[7] Einhorn, "Annalen d. Chemie," 1899.

[8] Braun, "Local Anesthesia," 1914.

[9] Sollman, *Jour. Pharm. and Exp. Ther.,* 11: 1, 9, 69, 159, 1918.

[10] Mulinos and Osborne, *Proc. Soc. Exp. Biol. and Med.,* 32: 1344, 1935.

[11] Rider, *Jour. Pharm. and Exp. Ther.,* 39: 329, 1930.

we demonstrated the ability of *epicaine* to produce definite sensory anesthesia within one minute in a ¼ 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 eye 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[1] stain (methylen blue and neutral red) or some staining mixtures proposed by later investigators, as, for example, Becquerel's[2] 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.

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 absorption (Luyet and Gehenio's[3] 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[4]).

BASILE J. LUYET

DEPARTMENT OF BIOLOGY
ST. LOUIS UNIVERSITY

[1] V. Ruzicka, *Pflügers Arch.*, 107: 473, 1905.
[2] P. Becquerel, *Comptes Rendus Ac. Sc.* 176: 601, 1923.

THE PREPARATION OF FINE FILAMENTS

THE special article entitled "Simplified Preparation of Microscope Cross Hairs"[1] 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.

JOSEPH B. FICKLEN

HARTFORD, CONN.

[3] 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.
[1] SCIENCE, 84: 490, 1936.