the microscope enables one to observe the structure of the crystals much better than is possible when a bright field condenser is used. The Stanley crystals were needle-shaped, appeared to have a granular structure and were admixed with a relatively small number of spheroidal particles. The "C" crystals were also needle-shaped, but were smooth in outline; this preparation appeared to contain fewer spheroidal particles than the Stanley preparation. The spheroidal particles were detected only when the dark field condenser was used. The optical activity of each preparation was determined; that of the Stanley preparation was found to be $[\alpha]_D^{22°}$ per mg nitrogen $= -38$, and that of the "C" preparation was -40. Stanley[2] reported the optical activity of his two samples of crystals to be -42 and -44. The fact that these 4 results are reasonably close, that the crystals are small needles similar to those of Stanley and that the virus concentration in these preparations is high indicates that these crystals are composed of the same material as those obtained by Stanley.

It was found that suspensions of the visible crystals in ammonium sulfate solution produced stream double refraction and that colloidal solutions of the crystals in buffers also showed this phenomenon. As much as 96 parts of buffer solution could be added to 1 part of the suspension of crystals before the solution reached the critical dilution. (The critical dilution is that dilution at which stream double refraction becomes undetectable.) By means of the first order red plate of the polarizing microscope it was found that the vibration direction of the slow ray of polarized light was always parallel to the direction of flow. This relation has also been observed in unpurified virus preparations. If these crystal preparations are pure virus, as many assume, it must be concluded that the virus can exhibit stream double refraction and, when in solution, is probably composed of submicroscopic rod-shaped particles.

Determinations were made of the critical dilution and active virus concentration in solutions of both of the crystalline preparations and in unpurified virus. The influence of pH on critical dilution and on active virus concentration was also determined. The number of local lesions produced on *Nicotiana glutinosa* L. was used as an indicator of active virus concentration. It was found that a given sample of virus produced approximately 14 per cent. more local lesions when at pH 7 than at pH 5.6. Conversely, a given sample of virus at pH 7 had a critical dilution approximately 36 per cent. lower than at pH 5.6. In other words, the number of local lesions produced was higher and the stream double refraction lower at pH 7 than at 5.6. These relations were found to hold for unpurified virus as well as the crystal solutions. As a hypothesis to explain this relation we would suggest that the virus is peptized at the higher pH and that more particles are therefore available to cause local lesions. To account for the lowered stream double refraction at pH 7 we would suggest that the refractive index of colloidal particles is probably lower at pH 7 than at 5.6; this should decrease the stream double refraction.

When stream double refraction is being used to determine the concentration of active virus the unknown and control should, as may be inferred from the above results, always have the same pH.

When solutions of crystals were diluted to the critical dilution the solution of Stanley crystals produced about twice as many local lesions as the solution of "C" crystals. When a critical dilution of unpurified control virus was compared with those of crystal solutions the solution of Stanley crystals produced an average of 24 per cent. less and the solution of "C" crystals 57 per cent. less lesions than did the unpurified virus. If the crystal preparations are pure, these results indicate that a significant portion of the virus in the crystals has become inactive during the purification process and that this inactivation is greatest in the "C" preparations.

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GENES AFFECTING RESPONSE OF NICOTIANA TABACUM HYBRIDS TO TOBACCO-MOSAIC VIRUS

IN *Nicotiana tabacum* L. and *N. paniculata* L. tobacco-mosaic virus (tobacco virus 1, *distorting* strain) causes a mottling-type disease, whereas in *N. rustica* L. and *N. glutinosa* L. the same virus causes necrosis. In a recent paper,[1] it was reported that a dominant gene, controlling necrotic type of response, was transferred from *N. rustica* to a self-fertile derivative of *N. paniculata* by hybridization of the two species, followed by repeated backcrosses to the recessive-type parent, and eventual self-pollinations. In the derived strain of *N. paniculata,* infection induced a necrotic-type, instead of a mottling-type, disease. This gene has now been carried from the necrotic-type strain of *N. paniculata* to plants of $[(N.\ paniculata \times N.\ tabacum) \times N.\ tabacum] \times N.\ tabacum$. There were considerable deviations from 1 : 1 ratios of necrotic-type to mottling-type plants in the backcross generations.

A similar gene for necrotic-type response has been transferred from *N. glutinosa* to three generations of hybrids with *N. tabacum.* All plants gave a necrotic-type response in the first generation hybrid *N. taba-*

[1] F. O. Holmes, *Phytopath.*, 26: 1007, 1936.

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.

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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.