

lated: C 46.26, H 3.43. Found: C 46.27, H 3.66. $[\alpha]_D^{25} = +48^\circ$ ($C = 0.125$ in pyridine). The natural thought at once occurred that this base might have a biogenetic relationship to alanine. Hydroxyisopropylamine (2-aminopropanol-1) was prepared by reduction of l-alanine ester with sodium in butyl alcohol. The resulting base gave a di-p-bromobenzoate (C 45.97, H 3.75), which melted at 155° and showed no depression with the above derivative obtained from the alkaloid. However, $[\alpha]_D^{25} = -41^\circ$ ($C = 0.415$ in pyridine). Therefore, the alkaloid amine is the natural form corresponding to d-alanine.

The alkaloid obtained by us (the ergobasine of Stoll and Burekhardt) is therefore the hydroxyisopropylamide of lysergic acid. In the formation of this substance, as in the case of the other ergot alkaloids studied by us, amino-acids or their derivatives are involved. It will be of interest to see what relationship to this alkaloid ergotocin will be shown to possess. Kharasch and Legault have given for it a formula $C_{21}H_{27}O_3N_3$, which thus differs from the former by C_2H_4O (or C_2H_5OH , alcohol?). The analyses of ergometrine reported by Dudley and Moir were made with the alkaloid recrystallized from benzene and could possibly have contained solvent.

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A SEROLOGICAL ESTIMATE OF THE ABSOLUTE CONCENTRATION OF TOBACCO MOSAIC VIRUS

ROBBINS¹ has assumed the molecular weight of tobacco mosaic virus to be in the neighborhood of 100,000, judging by the filtration studies of Duggar and Karrer, Waugh and Vinson, and MacClement and Smith. On the basis of infectivity tests on *Nicotiana glutinosa*, Caldwell² has estimated the concentration of infective particles to be of the order of 3×10^7 per cubic centimeter. If these two assumptions are correct, according to Robbins's calculations, the concentration of tobacco mosaic virus is such that 1 milligram is contained in 200,000 liters of expressed juice.

The most highly antigenic substance known, according to the serological literature, is the soluble specific carbohydrate of *Pneumococcus*, which gives a positive precipitin test with immune serum at about 1:2,500,000 (= .0004 milligrams per cubic centimeter). The most highly antigenic protein known is egg albumin, which gives a positive precipitin test at about 1:250,000 (= .004 milligrams per cubic centimeter). The writer has submitted evidence indicating that expressed saps of mosaic-diseased tobacco plants give precipitin titers

of about 1:250,³ and that it is the tobacco mosaic virus itself which is the antigen involved in this reaction.⁴

If tobacco mosaic virus sap is no more antigenic than the most highly antigenic substance known, the *Pneumococcus* carbohydrate, and if the minimal precipitating concentration of *Pneumococcus* carbohydrate is .0004 mg/cc, then it follows, *ipso facto*, that 1 cubic centimeter of tobacco mosaic expressed sap contains at least 0.1 milligram of virus (.0004 milligram \times 250).

One might question the advisability of comparing the serological behavior of tobacco mosaic virus, which is entirely specific, with that of the *Pneumococcus* carbohydrate, which exhibits a striking lack of source-specificity. The serological behavior of egg albumin is more typically comparable with that of tobacco mosaic virus. Comparable precipitin tests of egg albumin and tobacco mosaic virus have been performed by the writer, and the results gave a precipitin titer of 1:250,000 for egg albumin. Comparing the precipitin titer of tobacco mosaic virus with that of egg albumin, one finds that if tobacco mosaic virus is no more antigenic than egg albumin, 1 cubic centimeter of virus sap would contain at least 1 milligram of virus antigen. From the serological results with tobacco mosaic virus and on the basis of the premises stated above, it is hence estimated that the concentration of virus in expressed virus sap is no less than 0.1–1.0 milligram per cubic centimeter.

Furthermore, it is known that crude tobacco mosaic sap when diluted to 1:1,000,000 gives approximately one necrotic lesion per leaf on *Nicotiana glutinosa*, and that about 0.1 cubic centimeter of sap dilution is used in making the inoculation. From these facts, and assuming that Robbins's hypothesis of a molecular weight of 100,000 is correct for tobacco mosaic virus, it would follow that a cubic centimeter of virus sap contains 6.06×10^{14} to 6.06×10^{15} molecules of virus, and that a single minimal infective dose on *N. glutinosa* corresponds to 60–600 million molecules of virus antigen. The enormous ratio of 60–600 million virus molecules to a single infection, if our premises are correct, may be due to (1) a great loss of opportunity to infect in the cases of the myriads of virus particles which fail to fall in a position suitable for infection, *i.e.*, on the naked protoplast exposed in the breaking of a leaf hair, and (2) a possible aggregation of particles such that many antigenic molecules may coalesce, thus greatly reducing the actual number of discrete infective units. KENNETH STARR CHESTER

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¹ William J. Robbins, *SCIENCE*, 80: 275, 1934.

² John Caldwell, *Ann. Appl. Biol.*, 20: 100, 1933.

³ Kenneth S. Chester, *Phytopath.* (in press).

⁴ *Ibid.*