that which appears in the ratios of the helical lengths in the various organisms to that of the poliomyelitis virus. In each case it is close to being a whole number. This may be just a coincidence, particularly in the case of the *Proteus* helix, and it might be expected that its secondary helix, which has as yet not been resolved, would be the structure that would more probably be analagous to the viral helices. If this situation is actually a coincidence, it must be a rare one, for the probability of the 5 ratios all falling within two tenths of an integer by chance is of the order of 10^{-4}. On the other hand, if the results are not accidental, they suggest that the helices of the viruses and possibly also those of the larger organisms may be made up of multiples of some basic unit that is about 1500 A long. It appears significant that only the DNA-containing viruses have thus far shown these helical structures. Lengths of DNA molecules have been estimated to be in the range 4,500–9,600 A[10,11]. Of course, molecules of considerably greater length than 1500 A could be contained within the hypothetical unit mentioned above if they were coiled or folded. However, the observations suggest the possibility that the orientation of the DNA molecules may not be in a position parallel to the long axis of the fiber constituting the helices.

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The biosynthesis of the pyrrolidine ring of nicotine*

It has been suggested by ROBINSON[1] that the amino acid ornithine might be a precursor for the pyrrolidine ring of nicotine in tobacco plant metabolism but no conclusive evidence has ever been presented to substantiate this suggestion. KLEIN AND LINSER[2] were able to show an increase in nicotine content of tobacco plants when solutions of proline and ornithine were injected into the stems of the plants, but it was not clear whether ornithine had actually entered the nicotine molecule or had merely stimulated metabolism. In the experiments to be described in the present communication it was shown, using ornithine-2-^{14}C, that part of the ornithine molecule is incorporated into the pyrrolidine ring of nicotine.

Two groups of about 40 tobacco plants (*Nicotiana rustica*) were administered ornithine from a nutrient solution the composition of which has been described previously[3]. Prior to the hydroponic feeding of the amino acid the roots were removed from each plant and new roots were allowed to develop in the nutrient medium for a two week period. This experimental technique was employed since DAWSON[4] had demonstrated that nicotine is synthesized in growing roots. Each plant was then fed 1.5 μmoles of DL-ornithine-2-^{14}C hydrochloride** (0.25 mg) having a radioactivity of $4 \cdot 10^5$ counts per minute. All counts were made in an internal gas-flow counter and were corrected for self absorption. At the end of 5 days a similar quantity of the amino acid having the same radioactivity was again administered to each plant. Nine days following the second feeding of ornithine the plants were removed from the nutrient solution and the nicotine was isolated as the dipicrate as previously described[3]. The nicotine dipicrate possessed sufficient radioactivity so that it could be mixed with 9 parts of non-radioactive nicotine dipicrate to obtain enough material for the degradations described below.

For degradation of the pyrrolidine ring the nicotine was isolated from the dipicrate by an azeotropic distillation from a sodium hydroxide solution. The distillate was treated with aqueous permanganate according to the method of LAIBLIN[5] to oxidize nicotine to nicotinic acid. The MnO_2 formed by reduction of the permanganate was filtered from the oxidation mixture and the filtrate evaporated to dryness under reduced pressure. The residue was acidified with dilute nitric acid,

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** Purchased from Tracerlab, Inc. Boston, Mass.

and the liberated CO_2, which presumably arose solely from the 3, 4 and 5 positions of the pyrrolidine ring of nicotine, was bubbled into a saturated solution of barium hydroxide. The resulting barium carbonate was collected, washed, dried and counted for radioactivity. The nitric acid solution of the nicotinic acid was neutralized with aqueous ammonia and the silver salt of nicotinic acid was precipitated by addition of a silver nitrate solution. The nicotinic acid was liberated from the silver salt by treatment with hydrogen sulfide, and the free acid was recrystallized from water, dried and counted for radioactivity. There was no depression in melting point when a sample of this acid was mixed with an authentic sample of nicotinic acid.

Since the nicotinic acid was radioactive, it was decarboxylated by heating with calcium hydroxide according to WEIDEL's procedure[6] and the pyridine which distilled over was collected in a solution of methanol saturated with picric acid. The resulting pyridine picrate was collected and recrystallized from water.

Anal. calc. for $C_{11}H_8N_4O_7$: C, 42.86%; H, 2.60%; N, 18.18%.
found: C, 42.94%; H, 2.51%; N, 18.03%.

The pyridine picrate was counted and was found to contain no radioactivity. The carboxyl carbon of the nicotinic acid was recovered from the calcium carbonate in the distilling flask by acidification of the residue with dilute nitric acid and the liberated CO_2 was precipitated as barium carbonate by bubbling into a saturated barium hydroxide solution. The barium carbonate was collected, washed, dried and counted for radioactivity.

The possibility that some of the radioactivity of the nicotine might be located in the N-methyl group was investigated by demethylation of a sample of the nicotine according to a method previously described[3] whereby the methyl group was recovered as methyltriethylammonium iodide which is a suitable compound for counting.

From the data presented in Table I, which are the average of two separate experiments, it can be seen that approximately half of the radioactivity of the nicotine synthesized by plants fed ornithine-2-^{14}C was recovered in the nicotinic acid from the permanganate oxidation. All of this radioactivity in the nicotinic acid was located in the carboxyl group and none was present in the pyridine ring. Therefore, about half of the carbon-14 in the original nicotine was located in the 2-position of the pyrrolidine ring.

TABLE I

LOCATION OF RADIOACTIVITY IN THE NICOTINE MOLECULE AFTER THE ADMINISTRATION OF ORNITHINE-2-^{14}C

Compound	*Maximum specific activity (cpm/mM × 10^{-3})*
Nicotine dipicrate	15.2
Nicotinic acid	7.12
$BaCO_3$ (from nicotine permanganate oxidation)	7.46
Pyridine picrate	0
$BaCO_3$ (from nicotinic acid decarboxylation)	8.21
Methyltriethylammonium iodide	0.33

If it is assumed that the N-methyl group of nicotine is lost as methylamine in the permanganate oxidation, and that only the 3, 4 and 5 positions of the pyrrolidine ring are recovered from the oxidation mixture as barium carbonate, it will be noted from the data in the Table that the remaining half of the radioactivity of the nicotine was located somewhere in these three positions of the pyrrolidine ring. Since the administered ornithine was labeled in the α-position with carbon-14, it seems logical to assume that the 5-position of the pyrrolidine ring might contain half the radioactivity. A method is now being devised in this laboratory to isolate the 5-position for counting, and the results of these experiments will be reported later.

Less than 2% of the total radioactivity of the nicotine was recovered in the methyltriethylammonium iodide. The α-carbon of ornithine, therefore, is a very inefficient precursor of the N-methyl group of nicotine.

From these results it can be concluded that ornithine does serve as a specific precursor of the pyrrolidine ring of nicotine. Furthermore, if the assumptions made above are correct, it seems necessary to postulate that some symmetrical intermediate, such as pyrrolidine, is formed in the conversion of ornithine to nicotine to account for the distribution of the carbon-14 found in the isolated nicotine.

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