Note on helical structures in some bacteria and viruses*

This note is intended to call attention to structures seen in electron micrographs of some bacterial viruses and a pleuropneumonia-like organism which appear to the writer to have a helical form although they have not been so described by the investigators who published the electron micrographs mentioned. In a previous report, helical structures in *E. coli* considered to be analagous to the chromonemata of higher plants and animals were described[1]. The electron micrographs of the *Proteus* "dwarf" show lobulations of the surfaces along the long axis of the cell which resemble the unresolved helices of the *E. coli* particles[2,3]. The immature forms of the *E. coli*-T bacteriophages do not appear to be closed rings or "doughnuts" but seem rather to be helices having somewhat more than 1 revolution[4,5] and the *Staphylococcus* K bacteriophage has a similar appearance[6]. Helical structures have been found in biological structures unrelated to chromosomes[7,8] but their presence in viruses is of particular interest in relation to the problem of the fine structure of chromosomes.

If the helical form of the structures in these organisms be accepted, it becomes possible to examine them further to determine whether in addition to general similarity in configuration they show any dimensional regularities. When the diameter, pitch and number of revolutions of the helix can be obtained the length may be calculated from the equation

$$S = [(d/2)^2 + p^2]^{1/2} \cdot 2\pi n$$

where d is the diameter, p is the pitch, and n the number of revolutions.

In the case of the *Proteus* dwarf and bacteriophage T_2 linear scales are given on the published reproductions so that the magnification can be checked[2,4]. With the T_4 and K bacteriophages no linear scale is given and the magnifications are stated to be approximate[5,6]. No linear scale is given with the poliomyelitis virus electron micrographs but both the magnification and the absolute size are stated[9]. A series of measurements of arrays of a total of 49 particles made along 3 axes of the plaque gave an average length per particle in the illustration of 2.0 mm. The size of the particles as given by the authors was 27 mμ from which the magnification could be calculated and found to be 74,074 diameters, in good agreement with the 74,000 given with the illustration. Measurements were made with a steel rule with engraved markings at 0.5 mm with the aid of an aplanatic magnifier giving an enlargement of 7 diameters.

Using the same technique, measurements were made of the diameter and pitch of the helices in those particles in which the helical structure seemed apparent. In the case of the poliomyelitis virus, diameters could be obtained on 10 particles and the pitch on 12, the mean diameter being 1.5 mm and the mean pitch 0.5 mm. For the other organisms, with the exception of the *E. coli* chromosomes, only 2–6 measurements could be made. Because of the crudeness of the technique, and the limited number of measurements possible, the dimensions obtained can be assumed to be only approximations. It is therefore rather surprising to find the regularities which appear in the results shown in Table I.

TABLE I

	Dimensions of helices, in 10^{-6} cm				Relative
	d	p	n	S_x	S_x/S_p
1. *E. coli*, primary	30.0	17.1	5.7	848	—
secondary	4.8	8.0	106.0	5,562	370.8
2. *P. morganii*, primary	33.0	10.0	2.5	303	20.2
secondary	4.2	—	—	—	
3. Bacteriophage T_2	8.5	5.5	1.25	45	3.0
4. Bacteriophage T_4	6.8	4.8	1.25	46	3.1
5. Bacteriophage K	10.0	6.6	1.5	78	5.2
6. Poliomyelitis virus	2.0	0.7	2.0	15	1.0

d = diameter, p = pitch, n = number of revolutions, S_x = length of helix in 10^{-6} cm, S_x/S_p = length of helix relative to that of the poliomyelitis virus.

Source of measurements: 1. Ref.[1]. 2. Ref.[2], Fig. 12; Ref.[3], Fig. 10. 3. Ref.[4], Figs. 4, 4A, and 6. 4. Ref.[5], Fig. 4; Ref.[6], Figs. VIII 32 and 34. 5. Ref.[6], Fig. 30. 6. Ref.[9], Fig. 1.

The diameters of both the primary and secondary coils of the *A. coli* chromosomes and the *Proteus* "dwarf" are very similar in size which is to be expected if as TULASNE has suggested the "dwarf" is little more than a free chromosome[2]. The most striking and unexpected relationship is

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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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