

In the normal biosynthetic scheme the derivative would probably occur phosphorylated as proposed for intermediates in histidine auxotrophs of *Neurospora*²². Absence of phosphate in the derivative formed by *tr-1* could be due to phosphorolytic action by phosphatases or hydrolysis during the isolation procedure.

A mechanism of indole synthesis which incorporates the N-fructosyl-acid, and evidence available from the literature, is proposed as follows. N-(2-Carboxyphenyl)-1-amino-fructose-6-phosphate could arise from a direct condensation of anthranilic acid and fructose-phosphate. Glucose-phosphate could supply the carbohydrate unit with the enol or keto form resulting from an Amadori-type rearrangement²³ of the glucose unit. Decarboxylation and dehydration results in ring closure to form indole tetrose-phosphate. Elimination of tetrose-phosphate would yield free indole.

Further work is in progress to test the proposed scheme and more precisely define the indole-synthesizing reactions in *Saccharomyces*.

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Isolation and properties of ribosenucleic acid from tobacco leaves*

There is a considerable amount of work on the isolation and analysis of nucleic acids from animal and bacterial sources. The information on the nucleic acids of plant tissues is meagre. Pentose nucleic acid was isolated from wheat embryo by OSBORNE AND CAMPBELL¹ and LUSENA² and from barley roots by TAKASUGI³. Nucleoprotein was isolated from normal tobacco leaves by PIRIE⁴ and EGGMAN, SINGER AND WILDMAN⁵. HOLDEN AND PIRIE⁶ prepared nucleic acid from tobacco leaf nucleoprotein by three different procedures: (1) by adding an equal volume of 0.1 M NaCl at pH 8 and boiling the mixture, (2) by exposing nucleoprotein solution to 0.5 N NaOH for 40 minutes at 0° C and (3) by adding 50 g per liter trichloroacetic acid and keeping at room temperature for 8-12 hours. These procedures might yield degraded products. THOMAS AND SHERRATT⁷ estimated the purine and pyrimidine composition of nucleic acids extracted from

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leaves by 10% NaCl at 100° C. So far no attempt has been made to isolate ribosenucleic acids from plant leaves in an undegraded state and to characterize them. The present investigation deals with the isolation and characterization of ribosenucleic acid from normal tobacco leaves using a mild procedure. The purine and pyrimidine composition of the ribosenucleic acid (RNA) of tobacco leaves is also compared with that of the nucleic acid of tobacco mosaic virus grown in tobacco leaves.

Material. Leaves from healthy six weeks old Turkish tobacco plants, grown in a greenhouse were used.

Isolation. Unless otherwise stated all operations were carried out at 4° C. Immediately after picking, the tobacco leaves (350 g) were chilled for one hour and ground in a domestic meat-mincer. The minced material was mixed with 400 ml 0.1 M phosphate buffer of pH 7.1 and strained through a cheese cloth. The residue was again extracted with 200 ml buffer. The combined filtrates were centrifuged at 10,000 r.p.m. for 15 minutes. The pH of the supernatant was lowered to 4.5 with glacial acetic acid and was held at 0° C for one hour. The precipitate, separated by centrifugation, was triturated with 65 ml 0.14 M NaCl and pH was adjusted to 7.0 with *N* NaOH. The insoluble material was removed by centrifugation. The supernatant was added with stirring to three volumes of ethanol and was left overnight. The precipitate, obtained by centrifugation, was extracted twice with 0.1 M phosphate buffer of pH 7.1, using 12 ml for each extraction. To the extracts an equal volume of phenol saturated with water was added and the mixture was shaken for one hour. The aqueous phase containing RNA was separated by centrifugation and this process of deproteinization with phenol was repeated once again for 30 minutes*. The aqueous phase, freed of phenol by extraction with ether, was adjusted to pH 5.0 with glacial acetic acid, two volumes of ethanol were added and the mixture was left for one hour at 0° C. The precipitate, collected by centrifugation, was washed twice with 70% ethanol, dissolved in 5 ml distilled water and dialysed for 48 hours against several changes of distilled water. The dialysed material was lyophilized. Yield was 33 mg.

Properties. The RNA prepared according to the above procedure is a white powder, soluble in water. It gave a negative diphenylamine test for deoxyribose⁹ and a negative biuret test for proteins. The absorption spectrum, determined in distilled water by means of Beckman Spectrophotometer is given in Fig. 1. The values are plotted as $\epsilon(P)^{10}$. It exhibited an absorption maximum at 258 $m\mu$ and a minimum at 230 $m\mu$ with $\epsilon(P)$ values of 7485 and 3308 respectively. The $\epsilon(P)$ values for RNA of tobacco mosaic virus, prepared according to the procedure of COHEN AND STANLEY¹¹ as modified by KNIGHT¹², were 9350 at 258 $m\mu$ and 3345 at 230 $m\mu$.

Purine and pyrimidine composition. Purines and pyrimidines were estimated according to the procedure of WYATT¹³. The results are given in Table I.

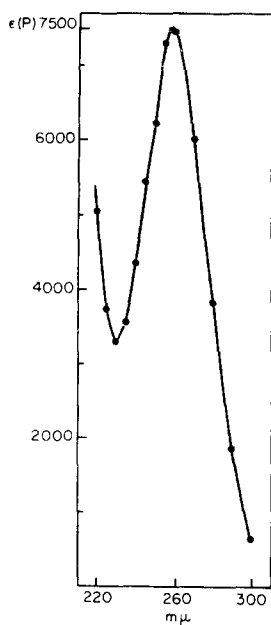


TABLE I
MOLAR RATIOS OF PURINES AND PYRIMIDINES OF RIBOSENUCLEIC ACIDS
OF TOBACCO LEAVES AND TOBACCO MOSAIC VIRUS

Bases	Tobacco leaf RNA	Tobacco mosaic virus RNA*
Adenine	0.71	1.13
Guanine **	1.00	1.00
Cytosine	0.72	0.70
Uracil	0.59	1.11
Purines	1.30	1.18
Pyrimidines		

* RNA was prepared according to the procedure of COHEN AND STANLEY¹¹ as modified by KNIGHT¹².

** Guanine was taken arbitrarily as 1.00.

Fig. 1. Ultraviolet absorption spectrum of tobacco leaf ribosenucleic acid in distilled water.

The RNA of tobacco leaves is characterized by a low uracil and a high guanine content. The purine and pyrimidine composition significantly differs from that of tobacco mosaic virus RNA. The RNA in the present investigation may, however, come from several parts of the cell and represent an average of types each with a qualitatively different composition.

The tobacco mosaic virus RNA used in this investigation was a gift from Dr. C. A. KNIGHT.

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Studies on the intestinal absorption of glucose

For a considerable time it was believed that glucose is absorbed from the intestine without degradation, probably by a mechanism involving phosphorylation and subsequent dephosphorylation^{1,2}.

However, recent studies of glucose absorption in the rat *in vivo* and *in vitro* claimed to show that glucose is converted to an unidentified compound which was transported in the portal blood to the liver³. *In vitro* studies by WILSON⁴ demonstrated that, although some glucose was transported across the intestinal wall of the rat, considerable degradation to lactate also took place. Experiments using guinea pig intestines have shown that glucose may be transported across the intestine *in vitro*^{5,6}.

An attempt to demonstrate whether glucose is degraded when absorbed by the intestine has been made by feeding glucose-1-¹⁴C (5 ml of a 50% glucose solution containing 10 μ C ¹⁴C) to rabbits by stomach tube. Blood samples were subsequently removed from the ear vein and deproteinised by the method of SOMOGYI⁷. 20 mg of carrier glucose were added and glucosazone prepared by the method of GARARD AND SHERMAN⁸. The osazones were purified, their radioactivity measured, and then were degraded to the bis-phenylhydrazone of the mesoxalic aldehyde by the method of TOPPER AND HASTINGS⁹. The specific activity of the mesoxalic aldehyde, containing carbon atoms 1, 2 and 3 from the glucose, was compared with that of the osazone as shown in Table I which gives the results of a typical experiment. In this degradation, formaldehyde arises from carbon atom 6 of the glucose. This was isolated as the dimesone derivative and no radioactivity was found in this substance. (The standard deviation in counting this compound was $\pm 10\%$.)

TABLE I

Time blood sample removed after glucose ingestion (min)	Specific activity of osazone (cts/min/ μ mole)	Specific activity of mesoxalic aldehyde bis-phenylhydrazone (cts/min/ μ mole)	Maximum possible randomisation (%)
15	5.5 \pm 0.22 *	5.8 \pm 0.43	0 \pm 8
30	9.5 \pm 0.25	11.9 \pm 0.30	0 \pm 4
45	14.9 \pm 0.32	13.7 \pm 0.47	8 \pm 5
60	16.8 \pm 0.44	18.1 \pm 0.40	0 \pm 6
90	9.6 \pm 0.25	9.2 \pm 0.26	4 \pm 4

* Standard deviation.