cent. These recoveries are based on a minimum of one ash, two moisture, and two uranium analyses. Since the accuracy of the uranium determination alone is at best ± 4 percent (*5*), these analyses are all within the limits of analytic error. It has been found by Cuttitta (*6*) that dry-ashing of the samples discussed in this report leads to no loss of uranium. It is evident, therefore, that destructive distillation of these carbonaceous substances results in no appreciable volatilization of uranium.

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* Publication authorized by the Director, U.S. Geological Survey.

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Isolation of C^{14}-Labeled DPN and TPN by Paper Chromatography from *Lactobacillus plantarum*

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A number of methods have been developed for the isolation of diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN) (*1–4*). Most of the present methods, however, are rather laborious. Previously, in this laboratory, several attempts were made to obtain C^{14}-labeled DPN from *Torula cremoris* grown in a synthetic medium (*5*) to which was added C^{14}-nicotinic acid. The method of Sumner, Krishnan, and Sisler (*3*) was used to obtain the DPN. Yields were extremely small, and purity of the compound was questionable. During studies of inhibition of spleen diphosphopyridine nucleotidase by nicotinamide, Zatman, Kaplan, and Colowick (*6*) obtained labeled DPN from C^{14}-nicotinamide and unlabeled DPN in the presence of spleen enzyme. The radioactive product was isolated and analyzed by ion exchange and paper chromatography. Although the exchange reaction was essentially 100-percent complete, the method was more involved than the one used in the present study (*7*).

In the process of studying the uptake of C^{14}-nicotinic acid and its amide by *Lactobacillus plantarum* (arabinosus) 17-5 (ATCC No. 8014) and several other organisms, it was found by Woodward and Boone (*8*) that about 50 percent of all the nicotinic acid or its amide present in the medium was adsorbed on the cell surfaces. Fifty percent uptake was obtained regardless of the amounts of C^{14}-nicotinic acid or its amide (up to the point of cell saturation) present in the medium and regardless of whether the cells were actively growing or were mature cells suspended in normal saline.

The C^{14} activity on the cells could *not* be removed by several washings with saline and distilled water. It was possible, however, to remove essentially all the radioactive material by repeated washings with *p*H 4 buffer. The primary purpose of this study was to determine whether the labeled material removed from the cell surfaces was unchanged nicotinic acid and/or the amide or some derivative.

Kodicek and Reddi (*9*) described a sensitive technique for the detection of nicotinic acid derivatives by chromatography. This technique was applied to the radioactive material washed from the cells. The use of *p*H 4 buffer as an eluting agent was unsatisfactory because the salt concentration, upon evaporation of the solution, interfered with the chromatography. Suspending the cells in distilled water and adjusting to *p*H 4 was tried and was equally as effective as the buffer for removing the radioactivity without disrupting the cells. Acidic washings were obtained from two groups of cells.

1) "Growing cells." *Lactobacillus plantarum* was grown at 37°C for 18 hr in a modified Snell and Wright medium (*10*) (used for microbiological assays of nicotinic acid) to which had been added 1 μg of C^{14}-nicotinic acid per 10 ml of medium. The labeled nicotinic acid had a specific activity of 4.07 mc/millimole which was equivalent to approximately 43,000 c/min, per 10 ml of medium, when counted in a gas flow proportional counter. At the end of 18 hr the cells were washed twice with 10 ml of 0.85 percent saline and once with distilled water. The cells were then suspended in 10 ml of distilled water, adjusted to *p*H 4, and allowed to stand for 15 min before centrifuging. Control cells treated in the same manner and counted by direct plating showed a C^{14} uptake of about 20,000 c/min. The acid eluate from the cells grown in two tubes of medium was pooled and evaporated to dryness at room temperature. This material was resuspended in 0.5 ml of distilled water. A small aliquot was taken for assay by direct plating, and the remainder (equivalent to about 40,000 c/min) was placed on filter-paper strips for chromatographic analysis.

This procedure was repeated using C^{14}-nicotinamide with a specific activity of 3.51 mc/millimole.

Descending chromatograms were run on the acid eluate from the cells using two solvent systems, n-butanol saturated with water and 60 percent n-propanol. Control samples of known C^{14}-nicotinic acid, C^{14}-nicotinamide, and nonlabeled DPN and TPN were chromatographed along with the acid eluate from the cells. The positions of the known DPN and TPN were located on the chromatograph strips by their characteristic fluorescence under ultraviolet light in the manner described by Kodicek and Reddi (*9*). The positions of the C^{14}-labeled compounds were established by autoradiographs of the filter-paper strips

prepared by placing them in contact with 14 × 17 x-ray film for 7 to 9 days.

The results of chromatographic analysis of the acid eluate from cells grown for 18 hr in the presence of C^{14}-nicotinic acid and amide are summarized in Table 1. Chromatograms of the acid eluate from the cells, using 60 percent n-propanol, showed two bands having R_F values of 0.15 and 0.26. These bands were located both by their radioactivity and by the characteristic fluorescence of DPN and TPN in the presence of ultraviolet light. The R_F values of the radioactive bands and the known samples of DPN and TPN were the same and were identical with those reported by Kodicek and Reddi (*9*) for known compounds of TPN and DPN. No material was left at the point of application, and no other metabolite was found in the acid washes. No traces of any remaining nicotinic acid or nicotinamide could be found in the acid eluate from the cells when either 60 percent n-propanol or n-butanol-water was used to develop the chromatograms. The radioactivity in the cell eluate and the samples of known DPN and TPN showed no movement from the point of application with n-butanol-water. All the radioactive material washed from the cell surfaces was apparently DPN and TPN. The DPN band was more intense and wider than TPN, both in radioactivity and fluorescence.

2) "Resting cells." *Lactobacillus plantarum* was grown in the same manner described in the preceding section except that 1 μg of nonlabeled nicotinic acid or amide was added per 10 ml of Snell and Wright medium. After 18 hr growth, the cells were washed as described in the preceding section and then were resuspended in 10 ml of saline that contained 1 to 2 μg of C^{14}-nicotinic acid or amide. The suspensions were incubated at 37°C for varying intervals of time, 15 min, 1 hr, and 18 hr. After incubation, the cells were washed and the radioactivity was eluted and chromatographed in exactly the same manner as that used for the "growing cells." The amounts of C^{14} activity eluted from the cells incubated for 1 and 18 hr were equivalent to those obtained from cells grown in the presence of the C^{14}-labeled compounds. The cells incubated for only 15 min showed a slightly lower uptake of C^{14} activity.

Table 1. Chromatographic analysis of acid eluate from cells of *Lactobacillus plantarum* grown with C^{14}-nicotinic acid and amide.

Solvent	Material	R_F value
60 percent n-propanol	C^{14}-nicotinic acid	0.72
	C^{14}-nicotinamide	0.84
	Known DPN	0.26
	Known TPN	0.15
	C^{14} cell eluate (2 bands)	0.26 and 0.15
n-Butanol-water	C^{14}-nicotinic acid	0.23
	C^{14}-nicotinamide	0.67
	Known DPN	No movement
	Known TPN	
	C^{14} cell eluate	

The chromatograms of the acid washes from "resting cells" that were suspended in saline containing C^{14}-nicotinic acid and/or amide showed results identical to those from cells grown in the presence of the labeled materials when n-butanol-water was used as the solvent.

When 60 percent n-propanol was used to develop the chromatograms, the acid washes from "resting cells" usually showed only one predominate radioactive band. This band had an R_F value of 0.26, identical with the chromatograph of known DPN and showed the characteristic fluorescence under ultraviolet light. This radioactive band occurred even in the washings from cells that were suspended in the presence of C^{14}-nicotinic acid for only 15 min, indicating that all the C^{14}-nicotinic acid or amide adsorbed on the cell surface was converted to DPN in a few minutes. Occasionally, a faint suggestion of the TPN band could be detected by both radioactivity and fluorescence. This band was not consistent and was extremely faint.

The specific activity of DPN was determined by eluting the labeled material from the chromatogram strips and diluting it to a known volume. A small aliquot of the sample was assayed for C^{14} activity and another for DPN by a modification of the microbiological method of Kohn and Bernheim (*11, 12*) using *Hemophilus parainfluenzae* (ATCC No. 7901). The results showed that the DPN had a specific activity of 3.90 mc/millimole compared with a specific activity of 4.07 mc/millimole for the C^{14}-nicotinic acid, indicating a direct stoichiometric relationship between the specific activity of the nicotinic acid in the medium and the DPN eluted from the cells. This relationship was also true when C^{14}-nicotinamide with a specific activity of 3.51 mc/millimole was used. Preliminary assay of the small amounts of TPN available using the same technique also indicated a stoichiometric relationship between the specific activity of C^{14}-nicotinic acid in the medium and TPN eluted from the cells.

This extremely rapid conversion of C^{14}-nicotinic acid to C^{14}-DPN by cells washed free from the growth medium and suspended in saline plus nicotinic acid indicates that all other moieties of the DPN molecule (phosphate, ribose, and adenine) must already be present in some form in the "resting cell." That an exchange phenomenon between unlabeled DPN and C^{14}-nicotinic acid is involved seems unlikely, since "resting cells" (upon being suspended in saline containing C^{14}-nicotinic acid) convert to DPN 10 to 20 times the amount of nicotinic acid supplied in the medium during their growth phase. Furthermore, the molar specific activity of the C^{14}-DPN, if formed by exchange, would approach only one-half of that of the C^{14}-nicotinic acid.

Quantitatively, 100 μg of C^{14}-nicotinic acid with a specific activity of 4.07 mc/millimole in the presence of 50 to 100 mg dry weight of cells of *Lactobacillus plantarum* (*13*) yielded about 250 to 300 μg of C^{14}-labeled DPN with equivalent molar specific activity.

Some, but not all, of the C^{14} activity remaining in the medium may be recovered by further cell absorption or as C^{14}-nicotinic acid or amide by ether extraction at the proper *p*H.

It is felt that considerable advantage may be offered by this method as a means of obtaining small amounts of pure C^{14}-labeled DPN and TPN with rather high specific activities. No attempt has been made in this preliminary paper to discuss cell surface adsorption phenomena. A discussion of this problem is being prepared by Woodward and Boone (*8*).

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Influence of Vitamins upon Incidence of Tumors in tu^{50j} Stock of *D. melanogaster*

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In previous reports, I (*1, 2*) have shown that the penetrance of tumor ^{50j} in *Drosophila melanogaster* is influenced by nutrition. In general, poor nutrition apparently lowers the incidence of tumors in the tu^{50j} stock. Tannenbaum (*3*) and Herskowitz and Burdette (*4*) also have found that poor nutrition and low caloric intake reduces the incidence of tumors.

An excess of several amino acids in the diet (*2*) increased the incidence of tumor in the tu^{50j} stock. This report is concerned with the influence of excessive amounts of vitamins on *D. melanogaster* reared on a rigorously controlled diet (*5*). A vitamin-free and amino acid-free medium (*2*) was inoculated with the yeast *Hansenula anomala,* so that the nourishment of the larva was restricted to the yeast employed. Excessive amounts of various vitamins were added to this medium, and the incidence of tumors produced by a pair of flies in a 10-ml vial was recorded. As the larvae burrow into the medium, some of the vitamins present are taken into the digestive tract.

The influence of the vitamins upon the presence of tumors in tu^{50j} stock of *D. melanogaster* is presented in Table 1. Note that the incidence of tumors is 10.6 percent in the flies reared on the minimal diet. However, significant increases in the tumor production result if the flies are reared on an excessive amount of riboflavin, nicotinic acid, pyridoxine HCl, vitamin B_{12} and p-amino benzoic acid. These vitamins, with the exception of p-amino benzoic acid, are included by Hinton (*6*) in a chemically defined diet of *D. melanogaster.*

Table 1. Tumor incidence in *D. melanogaster* tu^{50j} reared on various mediums inoculated with *Hansenula anomala.*

Added to 1 lit minimal medium		Flies	
Additive	(g)	Total No.	Tumorous (%)
None		2051	10.6
Ascorbic acid	0.333	760	10.1
Riboflavin	1.000	1153	14.8
Nicotinic acid	0.333	732	14.0
Pyridoxine HCl	.333	763	14.1
Calcium pantothenate	.333	781	12.5
Biotin	.333	767	9.1
Inositol	.333	715	10.5
Cholesterol	.500	712	9.2
Ergosterol	1.000	752	9.9
Calciferol	0.500	764	9.5
Vitamin K	.666	700	9.3
Vitamin A	.010	754	9.2
Thiamine HCl	.333	830	9.2
Vitamin B_{12}	.000010	746	21.6
Folic acid	.166	785	10.2
Choline HCl	.166	786	10.3
p-Amino benzoic acid	0.166	777	16.2

It appears that specific vitamins, as well as certain amino acids, in the diet of tu^{50j} stock increase the production of tumors. Thus, the cornmeal-molasses mixture which contains a higher percentage of vitamins and amino acids (*2*) does increase the penetrance of tu^{50j}.

These results present interesting questions that cannot be answered at present: (i) How do vitamins influence tumor production? (ii) Why does an excess of vitamin B_{12} in the diet double the number of tumors in the stock? (iii) Is there a critical amount of nutritional factors in the diet necessary for tumor formation? However, it can be stated definitely that the better the nutrition, the greater the chance of exposing the larvae to nutritional factors that increase the tumor production in tu^{50j} stock of *D. melanogaster.*

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