by the typhoid Vi I preparation was duplicated by the phage grown on *E. freundii*. Many additional tests of these two phages have failed to produce a single divergent reaction. The serologic identity of these phages was established by reciprocal cross-neutralization tests with standardized serums.

The absence of Vi antigen in a phage-susceptible culture of *E. freundii* and in a paracolon bacterium was adduced from inability to demonstrate its presence in agglutination, immunization, and agglutinin adsorption experiments. Slide-agglutination tests of numerous colonies from each of these cultures, as well as from other similar phage susceptible cultures, were made with typhoid Vi serum and were always negative. One rabbit was immunized with lyophilized organisms of *E. freundii* 10; another received cells of a paracolon culture (92) that had been dried from absolute alcohol. Antigens prepared by either means had been shown previously to be effective in engendering Vi-antibody formation by Vi-containing bacteria. No Vi agglutinin could be demonstrated in these serums either by slide- or tube-agglutination tests using living suspensions of *S. typhosa* (2V and Vi I). Although no Vi antigen could be detected by agglutination and immunization experiments, it was necessary to determine whether the cultures that were susceptible to the Vi I phage had the power to bind Vi agglutinin. For this purpose, three different paracolon strains and one strain of *E. freundii* were employed singly to adsorb a Vi serum prepared with a Bethesda-Ballerup paracolon (107). None of the adsorbing strains was able to reduce the Vi titer of this serum as measured by its ability to agglutinate living broth cultures or *S. typhosa* 2V or Vi I before and after adsorption. The adsorbing strains were fully susceptible to the Vi I phage at the time they were used in the experiment.

The afore-described experiments demonstrate the futility of any attempt to establish an absolute correlation between the presence of any particular antigenic constituent of a bacterial cell and its susceptibility to phage. Phage adsorption is a complex process that may be most easily understood in terms of the complementary ionic atmosphere of the phage particle and the adsorbing surface (*10*). It is quite likely that Vi antigen, represented by the presence of certain chemical groupings at the cell surface, exerts its effect on specificity of Vi phages insofar as it contributes electrostatic configurations favorable for adsorption. Pronounced effects of both Vi and O antigen on the electrophoretic behavior of *S. typhosa* have been reported (*11*). Similar effects were also observed (*12*) in a study of the stability of different forms of the typhoid organism in buffer solutions at various *p*H levels. However, it appears that substances serologically unrelated to Vi antigen may mediate the attachment of Vi phages to the cell. In all cultures of *S. typhosa*, and in at least some cultures of *E. freundii* and related paracolons, phage lysis of Vi+ and phage resistance of Vi– forms in the same culture were observed regularly. Vi antigen appears to condition these cells to lysis by Vi phages.

The present observations have no significance for the routine typing of *S. typhosa* by means of specific Vi phages. They may, however, be quite significant for the problem of distribution in nature of Vi-like substances. There are indications that Vi phages other than phage I may have an affinity for some Vi-negative cultures.

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Effects of Destructive Distillation on the Uranium Associated with Selected Naturally Occurring Carbonaceous Substances*

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Little is known regarding the organo-uranium compounds or complexes that may exist in uraniferous coal, shale, or other naturally occurring carbonaceous substances.

During the course of geochemical studies on uraniferous coals (*1*), yields of oil, water, and char were determined by a Fischer assay method (*2*). Because of the nature of this technique, it was difficult to establish with certainty whether volatile uranium compounds had been formed during the course of the retort assay. To investigate this possibility, a number of naturally occurring carbonaceous substances have been subjected to dry distillation in small-scale glass apparatus. Experimental results were evaluated on the basis of material balances using accurate techniques for determining the uranium content of the original substances and of the chars obtained from them. A preliminary search of the literature has revealed no publications regarding the volatilization of uranium from such substances during dry distillation.

This work is part of a program undertaken by the U.S. Geological Survey on behalf of the Division of Raw Materials of the Atomic Energy Commission.

The following samples were chosen to provide a variety of carbonaceous substances; the uranium con-

Table 1. Analyses of substances prior to retorting.

Sample	Moisture (%)	Ash (%)*	Uranium*	
			In ash (%)	In original substance (%)
Chattanooga shale	†	75.5	0.011	0.0083
Subbituminous coal	11.8	11.6	0.012	0.0014
Coal from Chattanooga shale	2.3	1.27	2.58	0.033
Swedish kolm	0.87	20.8	1.83	0.38

* Dry basis.
† Analysis on predried sample.

tents ranged from approximately 0.001 to 0.4 percent.

Chattanooga shale (Upper Devonian). Sample was taken 0.94 to 2.44 ft below top of top black interval, Cannon County, Tenn.

Subbituminous coal. Sample was collected from the upper foot of the Luman No. 1 bed, at its outcrop near the center of sec. 28, T. 24 N, R. 95 W, Sweetwater County, Wyo. Geochemical and mineralogic reports on this coal have appeared (*1, 3*).

Coal from Chattanooga shale. Black, lustrous, brittle organic material, subsequently identified as coal by J. M. Schopf of the U.S. Geological Survey (personal communication), was collected in 1953 by L. C. Conant and T. Kehn, also of the Survey, from an outcrop approximately 15 mi north of Nashville, Tenn. Beds of similar material having a maximum thickness of 0.04 ft are sparsely scattered in the shale averaging 3 to 5 seams per 30-ft core.

Swedish kolm. Lenticular specimens of kolm were collected at Kvantorp, Sweden, in 1951, by Breger.

The following techniques were used to obtain the data shown in Table 1 for the original samples and in Table 2 for the chars obtained from them:

Dry distillation. Samples of approximately 1 g were retorted, using a modification of the procedure suggested by Cuttitta (*4*). The material to be retorted was weighed in an 18- by 250-mm glass-stoppered Pyrex test tube. The lightly stoppered tube was then placed, sloping slightly down toward the stopper, in the tubular electric furnace where the material was distilled for 11 min at 500°C. After the tube was cooled in a horizontal position to prevent contamination of the char by the oil and water distillates, it was scored and broken at a point immediately above the char. The char was weighed and analyzed to determine its uranium content.

Determination of moisture. Weighed samples, approximately 0.5 g, of original material or char were dried in an oven for 2 hr at 105° ± 5°C. Loss of weight was recorded as moisture.

Determination of ash. Dry samples, approximately 0.5 g, were ignited to constant weight in an electric furnace at 800°C. Residual material was calculated as the ash content of the original dry sample.

Determination of uranium. Using the dry ash, the extraction-fluorimetric procedure described by Grimaldi, May, and Fletcher (*5*) was employed without modification.

Uranium balances based on the original samples and on the chars obtained from them are shown in Table 3; uranium contents have been calculated from the data of Tables 1 and 2.

The final column of Table 3 shows recoveries of uranium in the char ranging from 91.4 to 111.9 per-

Table 2. Analyses of chars.

Sample	Moisture (%)	Ash (%)*	Uranium*	
			In ash (%)	In original char (%)
Chattanooga shale	< 0.1	81.9	0.010	0.0082
Subbituminous coal†	2.4	19.0	0.016	0.0030
	2.4	18.8	0.016	0.0030
Coal from Chattanooga shale†	0.3	2.2	2.10	0.046
	0.4	2.4	1.93	0.046
Swedish kolm†	< 0.1	26.1	1.96	0.51
	< 0.1	26.1	1.96	0.51

* Dry basis.
† Duplicate analyses.

Table 3. Uranium balances.

Sample	Sample for distillation (g)	Uranium in sample (γ)*	Char (g)	Uranium in char (γ)*	$\frac{\text{Uranium in char}}{\text{Uranium in sample}} \times 100$ (%)
Chattanooga shale	0.9897	82.0	0.9150	75.0	91.4
Subbituminous coal†	0.9978	16.0	0.6034	17.9	111.9
	0.9858	15.8	0.5928	17.5	111.1
Coal from Chattanooga shale†	1.0019	320	0.6796	310	97.0
	0.9522	305	0.6349	305	100.0
Swedish kolm†	0.9784	3720	0.7811	3960	106.2
	1.0149	3860	0.8097	4130	107.0

* Micrograms.
† Duplicate analyses.

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.

References and Notes

* 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