might result from the possible introduction of adventitious organic matter in the substrate, inoculum, suspension medium, glassware, and connections in contact with the incubating cultures, plus controls to measure endogenous metabolism. The cultures, together with 25 mg. of hydrocarbon substrate, ignited sand, and sea water, were incubated at 32° C. for four days with the controls, during which time a stream of carbon dioxide-free air was bubbled through the culture receptacles at the rate of 50 ml./minute. The carbon dioxide evolved from the oxidation of the substrate and from endogenous metabolism was collected in N/10 NaOH during the incubation period. The residual carbon dioxide remaining in the solution after incubation was driven off by acidification. The endogenous control served also to eliminate the effect of carbon dioxide (as dissolved carbon dioxide, bicarbonate, and carbonate) present in the sea water other than that evolved by metabolic processes, which would be flushed into the caustic solution upon acidification.

The carbon dioxide collected at the receiving end of the apparatus was determined by back titration. That produced by the endogenous control was subtracted from the total

TABLE 1

AMOUNT OF CARBON DIOXIDE PRODUCED BY ACTION OF BACTERIA ON 25 MG. OF HYDROCARBONS IN FOUR DAYS AT 32° C.

Hydrocarbon	CO_2 produced (mg.)	Amount oxidized (%)
Naphthalene	44.2	51
Anthracene	53.5	64
Phenanthrene	58.5	68
Diaminobenzene	14.0	23
1,2-Benzanthracene	41.2	47
1,2,5,6-Dibenzanthracene	11.6	13
None (Control)*	0	—

* The controls used as checks against introduction of adventitious organic matter produced less CO_2 than the endogenous controls, hence are condensed as one control shown here.

carbon dioxide produced by the culture acting on the substrate to give the net amount of hydrocarbon oxidized by the organisms (Table 1). Since the empirical formulas and molecular weights of the hydrocarbons under test are known, the per cent oxidation of 25 mg. of the substrate was computed.

In view of the high oxidation rates, it does not seem unlikely that a quantitative oxidation of the carcinogenic hydrocarbons could be effected with a longer incubation period.

It is not contemplated that current investigations of the microbial utilization of carcinogenic hydrocarbons will continue beyond a brief survey of those compounds of particular structural interest. The findings from these cursory investigations have been presented with the hope of stimulating further research into the possible application of bacteria or their products in the prophylactic or therapeutic treatment of cancer.

References

1. HARTWELL, J. L. Survey of compounds which have been tested for carcinogenic activity. Washington, D. C.: U. S. Public Health Service, 1941.
2. ZOBELL, C. E. *Bact. Rev.*, 1946, **10**, 1.
3. ————. *Standard methods for the examination of water and sewage.* (9th ed.) New York: American Public Health Association, 1946.

Effect of Indole on the Determination of N′-Methylnicotinamide

B. S. SCHWEIGERT[1]

Department of Biochemistry and Nutrition, Agricultural and Mechanical College of Texas

The fluorometric method for determining the amounts of N′-methylnicotinamide in urine, which has been widely used, has been particularly valuable for measurements of N′-methylnicotinamide in the urine after the test animal has been fed tryptophane or related compounds (e.g. *4, 5, 6, 8*). In studies conducted in our laboratory (*6, 7*) measurements of N′-methylnicotinamide have been carried out without hydrolysis of the urine samples by the method of Huff, *et al.* (*2*). During the course of investigations on the effect of vitamin B_6 deficiency on the apparent conversion of tryptophane to nicotinic acid, it was of interest to determine the influence of feeding indole to rats on the amounts of N′-methylnicotinamide and nicotinic acid excreted in the urine.

TABLE 1

EFFECT OF FEEDING INDOLE TO RATS ON MEASUREMENT OF N′-METHYLNICOTINAMIDE AND NICOTINIC ACID IN THE URINE

(Values expressed as μg. excreted/rat/day)

Experimental series	Feeding regimen for indole					
	Before	During	After	Before	During	After
	N′-Methylnicotinamide			Nicotinic acid		
A	335	597	281	37	10	27
B	71	380	81	25	27	39
C	78	523	67	33	40	32
D	63	450	79	39	37	41
E	80	323	55	37	36	49
F	400	630	243	34	62	52
G	191	351	135	30	12	18
H	—	273	159	36	15	23
Average....	194	448	152	33.8	25.9	32.6

When a purified ration, adequate in vitamin B_6 but devoid of added nicotinic acid, was fed, the rats fed 100 mg. of dl-tryptophane/day excreted large amounts of N′-methylnicotinamide and nicotinic acid (*6*). However, when the rats were fed 50 mg. of indole/per day, a definite, though smaller, rise in N′-methylnicotinamide, but no increase in the amount of nicotinic acid, was observed. In fact, a slight reduction was noted in the average amount of the latter excreted (Table 1). This observation can be explained partially on the basis of a lower food consumption during the period when indole was added to the ration. In this work the same experimental regimen and analytical procedures were used as in earlier work (*6*).

These observations and those obtained on the ineffectiveness of indole in stimulating the growth rate of rats fed rations low in nicotinic acid (*3*; unpublished data) suggested that indole was not being utilized to form nicotinic acid derivatives, but was interfering with the fluorometric determination of N′-methylnicotinamide. To test this possibility, samples of urine from rats fed indole were extracted with ether to remove indole and some related compounds (*1*) prior to the estimation of

[1] The technical assistance of Patricia Sparks is gratefully acknowledged.

N′-methylnicotinamide. In addition, the effect of indole, tryptophane, and anthranilic acid on the measurement of N′-methylnicotinamide was determined, and the effect of indole was also tested in the presence and absence of urine. Some of these results are shown in Table 2.

Indole tested in the absence of urine showed some fluorescence measured as N′-methylnicotinamide. The fluorescence observed was equivalent to 12.2, 9.0, and 6.0 μg. of N′-methylnicotinamide when 1,000, 750, and 500 μg. of indole were tested. This effect could be eliminated by ether extraction of the indole solutions prior to the estimation of N′-methylnicotinamide. Anthranilic acid or tryptophane failed to show this interference, in that no fluorescence was observed when 1 mg. or more of these compounds was tested. It is readily apparent, therefore, that indole does interfere with the determination of N′-methylnicotinamide to a small extent, and this effect can largely be eliminated by the ether extraction procedure. Ether extraction of urine from rats fed indole reduced the values for N′-methylnicotinamide somewhat (Table 2), but did not reduce them to an extent whereby the

TABLE 2

EFFECT OF INDOLE ON THE APPARENT URINARY EXCRETION OF N′-METHYLNICOTINAMIDE BY THE RAT

(Values expressed as mg./ml. of urine)

Urine collection (No.)	Diet fed	Apparent excretion of N′-methylnicotinamide	
		Not ether extracted	Ether extracted
58	Basal ration	1.87	1.81
65	" "	4.02	4.00
66	" "	3.68	3.68
47	Basal + 50 mg. of indole/day	3.59	3.08
48	" " " " " " "	2.94	2.41
51	" " " " " " "	4.90	4.52
52	" " " " " " "	6.70	6.65
24	Basal ration + 100 mg. of dl-tryptophane/day	21.6	20.9
25	" "	20.1	19.8
26	" "	16.9	17.2
27	" "	16.2	17.9
24a	Basal ration + 100 mg. of dl-tryptophane/day + indole*	25.4	22.7
25a	" "	23.6	20.2
26a	" "	16.9	16.3
27a	" "	19.0	17.3

* In these tests 40 μg. of indole were added to each ml. of urine prior to estimations of N′-methylnicotinamide in each sample with and without ether extraction.

effect of feeding indole on the amounts of the methylated derivative found in the urine was negated. Control tests conducted with urine from rats fed the basal diet or basal diet plus tryptophane showed no reduction attributable to ether extraction of the samples. Similarly, N′-methylnicotinamide was not extracted by ether. Thus, it appears that extracting urine samples with ether removes only a portion of the interfering substances formed when indole is fed. The possibility, however, that when indole is fed some increase does occur in the amount of N′-methylnicotinamide excreted cannot be unequivocally ascertained. Furthermore, the large increases noted in the amounts of N′-methylnicotinamide and other nicotinic acid derivatives excreted when tryptophane is fed may be due, to a slight extent, to the presence of interfering compounds in the urine not extractable by ether. This possibility has also been recognized by other workers (*8*). No evidence has been obtained to suggest that indole or related compounds interfere with the microbiological determination of nicotinic acid. These observations indicate that the significance of the small rise noted in the amounts of N′-methylnicotinamide excreted when indole was fed is questionable.

Further work in identifying urinary nicotinic acid metabolites and refinements in methods for their estimation will be helpful in elucidating the metabolism of nicotinic acid and its relationship with other dietary nutrients.

References

1. GREENE, R. D., and BLACK, A. *Proc. Soc. exp. Biol. Med.*, 1943, **54**, 322.
2. HUFF, J. W., PERLZWEIG, W. A., and TILDEN, M. W. *Fed. Proc.*, 1945, **4**, 92.
3. KREHL, W. A., HENDERSON, L. M., DE LA HUERGA, J., and ELVEHJEM, C. A. *J. biol. Chem.*, 1946, **166**, 531.
4. ROSEN, F., HUFF, J. W., and PERLZWEIG, W. A. *J. biol. Chem.*, 1946, **163**, 343; *J. Nutrition*, 1947, **33**, 561.
5. SARETT, H. P., and GOLDSMITH, G. A. *J. biol. Chem.*, 1947, **167**, 293.
6. SCHWEIGERT, B. S., and PEARSON, P. B. *J. biol. Chem.*, 1947, **168**, 555.
7. SCHWEIGERT, B. S., PEARSON, P. B., and WILKENING, M. C. *Arch. Biochem.*, 1947, **12**, 139.
8. SINGAL, S. A., BRIGGS, A. P., SYDENSTRICKER, V. P., and LITTLEJOHN, J. M. *J. biol. Chem.*, 1946, **166**, 573.

Production of Starch-like Material From Glucose-1-phosphate by Diphtheria Bacilli[1]

EDWARD J. HEHRE, ARTHUR S. CARLSON, and JAMES M. NEILL

Department of Bacteriology and Immunology, Cornell University Medical College, New York City

This paper deals with the production of starch-like material from glucose-1-phosphate by *Corynebacterium diphtheriae* and some streptococci. The occurrence of starch-phosphorylases in yeast and in plant and animal tissues is well known, but, apart from our observation (*1*) that some strains of *Neisseria perflava* which form an amylopectin-like material from sucrose also form small amounts from glucose-1-phosphate, the capacity of bacteria to convert glucose-1-phosphate to products of the starch class has not previously been reported.

The bacteria tested in the present experiments included 17 strains of *C. diphtheriae* (12 had been recently isolated), 12 strains of streptococci Lancefield groups A to F, 9 strains of streptococci from endocarditis which form dextran from sucrose (*2*), and representatives of various other species of medical interest; for purposes of comparison, 3 strains of *N. perflava* were also included. The bacteria were inoculated into 3 mediums containing 2 per cent glucose-1-phosphate (crystal-

[1] This work was aided by a grant from the Sugar Research Foundation, Inc.