the 19 subjects of the 1940 outbreak showed any appreciable increase in antibodies against that virus. As shown in Fig. 2, the serums from the subjects of the 2 outbreaks reacted quite differently when tested against the *TM* virus; none of the 1939 group showed any appreciable increase in antibodies, whereas most of the patients of the 1940 group showed a significant rise in titer against that virus. These results indicate that the agent involved in the 1939 outbreak must have been either identical with or closely related to the *PR8*, and the agent involved in the 1940 outbreak either identical with or closely related to the *TM*. Thus, the data furnish an example in which the influenza that occurred in the one year was due to a virus distinctly different in antigenic properties from the virus responsible for the clinically similar disease that occurred in the same institution a year previously.

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Excretion of Nicotinic Acid in Pellagra.*†

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The impression is given by preliminary reports from several sources[1, 2, 3] that the excretion of nicotinic acid in pellagra is "not vastly different" from that of healthy individuals, in marked contrast to the excretion of certain other vitamins in their corresponding states of deficiency. This impression is fully confirmed by the results of the present report.

Observations have been made on several patients with deficiency diseases. Among these were cases of classical pellagra; others were states which were probably due to deficiency of several factors. Ob-

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1 Kühnau, W. W., *Klin. Woch.*, 1939, **18**, 1333.

2 Swaminathan, M., *Indian J. Med. Res.*, 1939, **27**, 417. (*Nut. Ab. Rev.*, 1940, **9**, 1037.)

3 Harris, L. J., and Raymond, W. D., *Biochem. J.*, 1939, **33**, 2037.

servations are also reported on a group of controls including normal individuals and patients without signs of any deficiency disease, and also 3 patients who were known to have had pellagra in the past.

Methods. Three types of nicotinic acid tolerance test have been employed: (1) The excretion of nicotinic acid in the urine after administration of a small (25 mg) dose by mouth. This was administered at midnight and urine collected from midnight to 6:00 a.m. The test was repeated on 2 successive nights and comparison made of nicotinic acid excretion over similar periods before and after; (2) The urinary excretion for 6 hours after administration of a large oral dose (200 mg nicotinic acid); (3) The urinary excretion for 3 hours after intravenous administration of 25 mg of nicotinic acid.

The avitaminotic patients were maintained on a pellagra producing diet. The three control cases who were known to have had pellagra in the past also received this diet. The other control subjects were unrestricted.

The determination of nicotinic acid was based on the color developed with cyanogen bromide and aniline, after acid hydrolysis, neutralization and clearing with zinc hydroxide. Throughout most of the study hydrolysis was extended for 2½ hours at 95°C with an acidity of 1½ N HCl. Following the appearance of the study of Melnick, Robinson, and Field[4] it was observed that the same results were obtained by hydrolysis for 30 minutes in boiling water with an acidity of 2½ N HCl. Thereafter the shorter procedure was employed, and the results as was pointed out may be taken to include nicotinic acid plus nicotinamid plus two-thirds of any nicotinuric acid which may be present. Zinc hydroxide was employed for removal of pigment at the suggestion of Dr. H. A. Waisman. It was found not to remove a significant quantity of nicotinic acid, and recovery experiments gave results consistently better than 90%. Nicotine was also found to escape removal by this precipitation and to possess about 70% of the color value of nicotinic acid.

Technic of color development was that of Pearson;[5] results were reproducible providing the interval between addition of cyanogen bromide and aniline was timed accurately to 2 minutes. The Evelyn photoelectric colorimeter with the 440 filter has been employed for measurement of color intensity. Since all of the urinary pigment was not removed by treatment with zinc hydroxide, a reading was taken immediately before development of color; the value of this

[4] Melnick, Daniel, Robinson, W. D., and Field, Henry, Jr., *J. Biol. Chem.*, 1940, **136**, 131.

[5] Pearson, P. B., *J. Biol. Chem.*, 1939, **129**, 491.

reading was diminished by the factor of dilution due to addition of aniline and cyanogen bromide and then subtracted from the value of the final reading.

Sodium citrate was added as a buffer, since the color is influenced by changes in acidity. The acidity of the final mixture with this technic is a little less than that of Pearson, and the rapidity of color development a little greater.

The nicotinic acid results in Exp. I were corrected for creatinine excretion; they were multiplied by the factor: average creatinine

TABLE I.

Tests of Nicotinic Acid Tolerance, Employing a 25 mg Oral Dose. Urinary Excretion mg Nicotinic Acid for 6-hr Period.

	Period						
	Fore		Test		After		
Control group	1st day	2nd day	1st day	2nd day	1st day	2nd day	
L.G.	.40	.36	.48	.37	.33	.28	Bronchial Asthma
G.G.	.23	.20	.39	.29	.16	.26	Rheumatic Heart Disease
J.R.	1.32	1.65	2.68	2.00	—	—	Arthritis
R.P.C.	.17	.24	.15	.83	.19	.31	Hypertension
W.E.	.29	.38	.46	1.51	.19	.29	Bacterial Endocarditis
C.S.	.57	.35	.29	.59	.57	—	Rheumatic Fever
I.G.	.22	.31	.33	.52	.29	.24	Arthritis—Healed Pellagra
W.J.	.73	1.06	1.27	1.15	1.09	1.02	Nephritis " "
K.G.	.41	.40	.33	.60	.28	.36	Hypertension " "
Dr.W.	1.29	1.34	1.55	1.46	1.72	—	Normal Control
Dr.S.	2.36	2.00	2.09	1.70	1.76	—	" "
Dr.B.	.32	.25	.32	.27	.21	—	" "
Max.	2.36	2.00	2.68	2.00	1.76	1.02	
Min.	.22	.20	.15	.27	.16	.26	
Mean	.69	.71	.86	.94	.62		
Deficient group							
Cl.	2.19	2.18	4.36	4.02	—	—	Pellagra—Active
Gl.	2.24	2.41	3.58	2.93	—	—	" "
M.W.	.06	.12	1.01	1.00	.31	.29	" "
Wi.	2.78	2.44	2.56	3.06	2.24	1.81	" "
Bl.	2.89	2.24	2.48	2.07	2.21	2.88	" "
K.H.	.21	.35	1.30	1.73	.38	.42	" "
Go.	1.01	1.11	2.03	1.77	—	—	" "
G.D.	2.67	2.73	2.98	2.14	—	2.75	" ?
W.S.	.54	.43	1.53	.94	.44	.45	" ?
P.N.	1.17	.68	.97	1.04	.77	.84	" ? Ariboflavinosis
Ne.	.26	.26	1.65	1.68	.36	—	" ? "
La.	.89	1.22	1.25	1.37	1.15	1.12	" Pit. Cachexia ?
Max.	2.89	2.73	4.36	4.02	2.24	2.88	
Min.	.06	.12	1.01	.94	.31	.29	
Mean	1.41	1.35	2.14	1.98	.98	1.32	

÷ (divided by) daily creatinine. Creatinine was determined with the aid of the photoelectric colorimeter and the Jaffe reaction.

Results. Chief interest attaches to the results of Exp. I. (Table I.) The basal 6-hour excretion of the control group in the fore periods is observed to range from 0.20 to 2.36 mg nicotinic acid. The equivalent 24-hr values of about 1 to 10 mg are of the same order as those which have been reported for complete 24-hr urines.[1-4]

The highest values in the control group were obtained from the subjects, Dr. W., Dr. S., and J. R. Of these Drs. W. and S. were known to smoke at night and the slightly higher results were thought to be due to nicotine. The other subjects of the control group, as well as those of the deficiency group, were instructed not to smoke after 6:00 P.M. Possibly, however, some of the results which seem to be out of line were also due to night smoking.

The basal 6-hour excretions of the 3 healed pellagrins, on a deficient diet, are not lower than those of some of the other members of the control group on unrestricted diets.

It was anticipated that a slight increase in excretion during the test period would be shown by the normal individuals and that the patients with deficiency states would show a lesser increase or perhaps none at all. A glance at the table reveals that nothing of the sort was realized; actually the opposite is more nearly true. The mean increase of the test period over the fore period in the deficiency group is about 0.6 mg, compared to an increase of about 0.2 mg for the control group. Also the mean basal 6-hour excretion of the deficiency

TABLE II.
Additional Tests of Nicotinic Acid Tolerance.

	25 mg Intravenous 3 hr Excretion	200 mg Oral 6 hr Excretion	
Control group			
Hu.	2.51	10.90	Nephritis, acute
Sm.	1.36	7.11	Cardiac
Fi.	1.03	7.88	Hypertension
J.S.		17.70	Cardiac
A.T.		8.87	Pneumonia
I.G.	1.15	12.25	Arthritis
Gr.	3.14	16.10	"
Jo.	1.93	4.92	Nephritis, chr.
Deficient group			
Bl.	4.33	9.30	Pellagra
K.H.	1.06	3.46	"
Wi.	5.25	18.74	"
W.S.	1.02	15.20	"
P.N.	1.46	16.61	? Ariboflavinosis
Ne.	1.19	12.52	? "

group is a little greater than that of the controls. However, the differences are not great and probably should not be considered significant, especially since other reports have generally observed slightly lower values for nicotinic acid excretion in the deficient state.[1, 2, 3]

The results of the other 2 tolerance tests are shown in Table II. Again the response of the deficient group is seen to be not significantly different from that of the control group.

It is of some interest to contrast the responses of 2 members of the deficient group (B. and H.). They had a similar pellagrous eruption; they were on the same deficient diet; and each smoked occasionally during the day. H. showed a low basal excretion and a poor response to all tests; B., on each occasion, gave relatively high values.

The similar behavior of the 2 groups must depend on similar levels of plasma nicotinic acid. Melnick, Robinson and Field[6] found a parallel between fluctuations of plasma (free nicotinic acid values) and urinary excretion. They also observed that the plasma level exhibited a tendency to remain constant; only transient fluctuations with nicotinic acid dosage were noted. Since there appears to be no significant difference in regard to the level of nicotinic acid in whole blood between the normal and deficient state,[7, 8] it might be expected to find the basal urinary excretion to be essentially unchanged in pellagra.

Summary. The observations reported here indicate that the urinary excretion of nicotinic acid in pellagra is not significantly different from that of the normal.

[6] Melnick, Daniel, Robinson, W. D., and Field, Henry, Jr., *J. Biol. Chem.*, 1940, **136**, 157.

[7] Ballif, L., Lwoff, A., Querido, A., Ornstein, I., *Comp. Rend. Soc. Biol.*, 1939, **131**, 903.

[8] Unpublished work in this laboratory.