Effect of Temperature on Oxygen Poisoning

Previous work had indicated that the lowering of the environmental temperature increased the oxygen tolerance of small experimental animals (de Almeida, 1934; Campbell, 1937). Three series at 50 ft. were performed, at 87.5° F. (30.8° C.) (steam-heated), at 45° F. (7.2° C.) (ice-cooled), and control exposures at 65° F. (18.3° C.). Divers were allowed to vary their underwear under the suit as the application of this work to operational problems was the first consideration. It is doubted if these exposures could have been tolerated without this variation in clothing. No exercise was carried out. This was advantageous at a higher temperature but disadvantageous to the diver in cold water. The divers complained bitterly of the cold and found the heated dives equally uncomfortable. These experiments showed that oxygen tolerance was equally affected by heat and cold. Although the performances below 30 minutes were only slightly impaired all outstanding performances were eliminated. It is possible that this delayed effect was due to changes in body temperature, as symptoms suggested that this occurred after about 20 minutes.

[To be concluded, with a bibliography, in next week's issue.]

THE NICOTINAMIDE SATURATION TEST

BY

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Nicotinamide saturation tests have been carried out by numerous workers (Holt and Najjar, 1943; Ellinger and Coulson, 1944; Ruffin, Cayer, and Perlzweig, 1944; Roberts and Najjar, 1944; Coulson, Ellinger, and Smart, 1945; Ellinger, Benesch, and Hardwick, 1945), based on the daily urinary elimination of nicotinamide methochloride and the response to orally administered nicotinamide. These tests are believed to provide information about the nicotinamide state of the tested person, and, indeed, the findings by Holt and Najjar (1943), Roberts and Najjar (1944), Ellinger, Benesch, and Kay (1945), and Ellinger, Benesch, and Hardwick (1945) show a significantly lower nicotinamide methochloride elimination and response to ingested nicotinamide in pellagrins than in physically fit persons. This is remarkable, since the intake, consumption, and storage of nicotinamide, which determine the nicotinamide state of the body, are probably not the only factors responsible for the extent of the elimination of nicotinamide methochloride. Ellinger and Coulson (1944) mentioned the presence of "methyl-donators" and the efficiency of the methylating mechanism as influencing the extent of nicotinamide methochloride elimination. Malabsorption from the intestines was earlier considered as a possible aetiological factor in pellagra (Ellinger, Hassan, and Taha, 1937), and it was proved to affect the response to ingested nicotinamide in pellagrins but not in healthy persons (Ellinger, Benesch, and Kay, 1945). The recent knowledge of the importance of the intestinal flora for the nicotinamide intake (Ellinger, Coulson, and Benesch, 1944; Ellinger, Benesch, and Kay, 1945; Najjar, Holt, Johns, Medairy, and Fleischmann, 1946), the fact that some intestinal bacteria

consume nicotinamide, and the findings by Koser and Baird (1944) that, *in vitro*, some strains of *Pseudomonas* and *Serratia*, and by Benesch (1945) that anaerobes from the intestines, also *in vitro*, destroy nicotinamide, suggest other factors influencing the response to orally administered nicotinamide. It was desirable to study the influence of these various factors on the response to ingested nicotinamide. Unfortunately the number of cases of various liver diseases was so far not large enough for study of the effect of the methylating mechanism, and experiments on rats with induced liver diseases were not yet conclusive (Ellinger, 1946).† But the effect on the response to ingested nicotinamide of the presence of "methyl-donators" and of the route of ingestion could be studied, and this forms the subject of the present communication.

Experimental

Experiment 1.—On 5 healthy persons (2 males, 3 females) and 1 diabetic male, balanced by diet and insulin, of known low methylating capacity (Nos. 1-6). The daily nicotinamide methochloride elimination was measured in 24-hour samples of urine for 3 days without extradietary intake of nicotinamide and, on the 4th day, after the oral ingestion of 100 mg. of nicotinamide at the beginning of the 4th day's urine collection. On the 5th, 6th, and 7th days no urine was collected. On the 7th to 11th days 1 g. of methionine was ingested orally every 12 hours. Urinary nicotinamide methochloride elimination was investigated in 24-hour samples on the 8th to 11th days; no extradietary nicotinamide was administered on the 7th to 9th days, and 100 mg. of nicotinamide was taken by mouth on the 10th day. The experiment was spread over the second half of 1945. All persons continued with their usual work and had their usual food during the experiment.

Experiment 2.—On 5 physically fit inmates of West Park Hospital—1 male and 4 females (Nos. 7-11). The urinary elimination of nicotinamide methochloride was estimated for 14 days—the first 3 days without extradietary administration of nicotinamide, the remaining 11 days after daily ingestion of 100 mg. of nicotinamide at the beginning of the collection period. In addition, 1 g. of methionine was ingested by mouth every 12 hours on the 9th, 10th, and 11th days. The experiment was carried out between March 14 and 31, 1945. All persons in this and the following experiments received the usual hospital diet and were kept in bed or indoors during the experiment.

Experiment 3.—On 8 inmates of West Park Hospital—1 male and 7 females (Nos. 12-19). Four of these were physically fit, one was a physically fit woman who had suffered from pellagra in recent years, and three were pellagrins. The only differences from Experiment 2 were that every second subject was given the daily 100 mg. nicotinamide subcutaneously instead of orally during the whole experiment, while the other half received it by mouth as all subjects in Experiment 2, and the nicotinamide methochloride estimation was continued for one (15th) day after the nicotinamide administration came to an end. The experiment was carried out from April 30 to May 23, 1945.

Experiment 4.—On 5 physically fit and 3 pellagrous female inmates of West Park Hospital (Nos. 20-27). The arrangements in this experiment differ from those of Experiments 2 and 3 in that nicotinamide was administered throughout the experiment to all persons subcutaneously instead of orally, and that 500 mg. (250 mg. twice daily) were given instead of 100 mg. for all 11 days. Urinary nicotinamide methochloride elimination was measured for 15 days, as in Experiment 3. The experiment was carried out between July 10 and 25, 1945.

Experiment 5.—On 9 inmates of West Park Hospital—3 males and 6 females (Nos. 28-36). Six of these were physically fit

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† Since this paper was submitted for publication experiments have been carried out showing that experimental liver poisoning in rats causes, after a preliminary rise in nicotinamide methochloride output due to tissue disintegration, a diminished spontaneous nicotinamide methochloride elimination in urine and a diminished response to dosed nicotinamide. Evidence has been obtained also for the elimination of nicotinamide methochloride in the bile and the destruction of the compound by intestinal bacteria *in vitro* (Ellinger, 1947).

and three were mild acute or chronic pellagrins. The urinary nicotinamide methochloride eliminations were measured for 15 days. After three days without extradietary nicotinamide administration 100 mg. of nicotinamide was given to each person at the beginning of the 4th, 7th, 10th, and 13th days of the collecting period, alternatively subcutaneously, orally, or rectally. Three people commenced with subcutaneous, three with oral, and three with rectal administration, and at the end of the course (13th day) ingestion by the first route was repeated; thus each person received ingestion by one route twice and by the other routes once. The experiment was carried out between Oct. 28 and Dec. 4, 1945.

On some occasions the same person was used for more than one experiment; these were Nos. 7, 12 (2); 8, 13 (4); 10, 14 (6); 9, 15, 22, 30 (5). Some of them had already been examined in the experiments reported by

administration of nicotinamide. The difference is multiplied as before by 100 and the molar weight of nicotinamide, and divided by the weight in mg. of the administered nicotinamide and the molar weight of nicotinamide methochloride.

Discussion

Experiments 1 to 4 (Tables I to IV) should be discussed simultaneously. The pre-dosing nicotinamide elimination of those people who have been examined more than once is remarkably constant over a fairly long period; only the values for the January experiment (Ellinger, Benesch, and Hardwick, 1945) were considerably higher in three instances. In one case—(6) 10, 14—the output varied considerably and was lower in January than on the later occasions. The pre-dosing nicotinamide methochloride

TABLE I.—Daily Urinary Elimination of Nicotinamide Methochloride (NM) and Response to Orally Dosed 100 mg. Nicotinamide (NA) Before and During Daily Oral Ingestion of 2 g. of Methionine

No.	Date	Sex	Age	Condition of Health	Daily NM Elimination on Pre-dosing Days (mg.)				Recovery* of NA in % of Ingested 100 mg. NA	Daily NM Elimination during Methionine Dosing (mg.)				Recovery* of NA (as NM) in % of 100 mg. Ingested NA during Methionine Dosing
					1	2	3	Aver.		1	2	3	Aver.	
1	16-26/7	F.	20	Fit	6.2	6.8	5.7	6.2	10.2	5.8	6.6	6.3	6.2	11.2
2	15-25/8	F.	52	Fit	5.3	6.0	5.7	5.7	11.7	5.0	5.7	6.0	5.6	10.7
3	3-13/9	M.	58	Diab.	3.8	4.1	3.7	3.9	6.2	3.4	3.7	3.3	3.5	5.2
4	17-27/9	M.	26	Fit	8.2	8.9	9.1	8.7	12.7	8.4	8.9	9.2	8.8	13.5
5	1-11/10	F.	24	Fit	3.6	3.4	3.9	3.6	10.8	3.9	4.2	4.4	4.2	8.9
6	5-15/11	M.	32	Fit	7.3	7.9	8.2	7.8	15.8	6.8	7.2	7.3	7.1	14.9
Average:					5.7	6.2	6.1	6.0	11.2	5.6	6.1	6.1	5.9	10.7

*Urinary NM in mg. — pre-dosing NM average in mg. $\times \frac{\text{mol.W. of NA}}{\text{dosed NA in mg.}} \times 100$

TABLE II.—Response of Physically Fit Persons to Oral Application of 100 mg. of NA Before, During, and After Daily Oral Dosage of 2 g. of Methionine (March 14-31, 1945)

No.	Sex	Age	Weight		NM Elimination in mg. on Pre-dosing Days				Recovery of NA (as NM) in % of Ingested NA*															
									On 100 mg. NA Dosing Days								On 100 mg. NA-2 g. Methionine Dosing Days				On 100 mg. NA Dosing Days			
			lb.	kg.	1	2	3	Aver.	1	2	3	4	5	Aver.	1	2	3	Aver.	1	2	3	Aver.		
7	M.	34	214	97.2	5.20	—	5.07	5.14	—	14.2	17.1	17.3	17.7	16.6	14.2	13.8	11.1	13.0	11.4	42.6	72.5	42.8		
8	F.	40	122	55.3	4.75	5.26	5.09	5.03	9.8	12.4	14.3	13.8	15.6	13.2	12.9	6.1	58.2	25.7	52.8	82.4	85.9	73.7		
9	F.	23	142½	64.8	5.14	—	3.18	4.16	6.7	14.9	14.6	8.3	7.3	10.2	9.8	8.6	77.6	32.0	73.0	28.5	38.2	46.6		
10	F.	54	136	61.8	3.95	4.56	4.58	4.36	11.5	16.4	17.5	28.5	12.5	17.3	12.0	12.8	76.8	33.9	119.0	32.9	71.4	74.4		
11	F.	34	85	38.6	—	4.28	—	4.28	7.3	12.8	7.3	11.4	11.3	10.0	1.9	7.2	4.7	4.6	0.9	39.7	25.2	21.9		
Average:					4.76	4.70	4.48	4.62	8.8	14.7	14.2	15.7	12.9	13.5	10.1	9.9	45.7	21.9	51.4	45.1	58.6	52.1		

* See note under Table I.

Ellinger, Benesch, and Hardwick (1945). The figures in brackets indicate their numbers in that paper. In all cases collection of urine was done in the same way as described by those authors. The daily urinary nicotinamide methochloride output was measured by the method of Coulson, Ellinger, and Holden (1944).

Results

The results of Experiments 1-5 are shown in Tables I-V respectively. In all tables the pre- and post-dosing elimination of nicotinamide methochloride is presented in mg. per day, while the response to ingested nicotinamide—that is, the recovery of nicotinamide—is given as percentage of the ingested nicotinamide. This is calculated by deduction of the daily pre-dosing average elimination of nicotinamide methochloride in mg. from the elimination in mg. during 24 hours after dosing nicotinamide. This difference is multiplied by 100 and the molar weight of nicotinamide, and divided by the mg. of nicotinamide dosed and the molar weight of nicotinamide methochloride. In Table V the recovery for 72 hours after dosing nicotinamide is found by deducting three times the daily pre-dosing average elimination in mg. from the sum in mg. of the daily elimination in mg. for three days following the

elimination was in all instances considerably lower in the pellagrins than in physically fit persons.

The response to a five-day course of 100 mg. of nicotinamide was less constant in the fit persons examined more than once, but always much above that of the pellagrins. The response to a daily intake of 500 mg. of nicotinamide was not only absolutely but also relatively considerably above that to a daily intake of 100 mg. The relative response to 500 mg. was even more increased in pellagrins than in fit persons, so that the difference between pellagrins and non-pellagrins was far less distinct than after the intake of 100 mg. A test with 100 mg. of nicotinamide therefore provides a more significant result than one with 500 mg.

So far there has not been much evidence that the presence or absence of "methyl-donators" might influence the extent of nicotinamide methylation and consequently control the height of urinary nicotinamide methochloride elimination. Perlzweig, Bernheim, and Bernheim (1943) observed that in some cases the addition of methionine increased the formation by rat-liver slides of nicotinamide methochloride from nicotinamide. In confirming these results Ellinger (1946a) found that methionine increases the methylation of nicotinamide by slices of liver of those rats

TABLE III.—Response of Physically Fit Persons and Mild Pellagrins to Oral or Subcutaneous Application of 100 mg. of NA Before, During, and After Daily Dosage of 2 g. of Methionine (April 30 to May 23, 1945)

No.	Sex	Age	Weight		Way of Admin.	Clinical Diagnosis	NM Elimination in mg. on Pre-dosing Days					Recovery of NA (as NM) in % of Ingested NA *										NM Elimination in mg. on Post-dosing Day 1						
			lb.	kg.			On 100 mg. NA Dosing Days					On 100 mg. NA-2 g. Methionine Dosing Days					On 100 mg. NA Dosing Days											
							1	2	3	Aver.	1	2	3	Aver.	1	2	3	Aver.										
12	M.	34	214	97.2	Oral	Physically fit	5.56	6.65	4.96	5.72	10.9	12.5	7.6	10.7	6.9	15.5	13.6	12.0	11.8	10.9	13.9	16.6	13.8	16.7	10.6	8.9		
13	F.	40	122	55.5	Sub-cutaneous	"	4.80	6.20	4.64	5.21	10.6	9.2	11.1	13.8	17.2	11.4	2.8	10.6	11.4	11.4	14.3	26.8	19.5	7.2	29.6			
14	F.	54	134	60.9	"	"	7.28	6.95	7.60	7.21	3.8	23.5	11.3	22.0	18.7	19.6	23.1	20.5	17.7	6.5	27.2	17.1	18.7	—	—			
15	F.	23	142½	64.8	"	"	4.17	4.44	4.15	4.25	13.3	12.9	11.5	17.3	14.5	14.0	13.5	15.8	18.8	20.3	21.8	20.3	—	—	—			
Normal controls; average:							5.36	6.01	5.28	5.50	9.7	14.5	10.3	14.5	13.0	14.3	15.0	14.7	14.7	14.7	13.7	23.0	17.1	15.8	—			
16	F.	55	144	65.5	Sub-cutaneous	Physically fit at the time; pellagra in recent years	2.26	3.14	2.81	2.74	11.0	5.7	8.5	10.9	12.1	10.1	13.6	20.0	14.5	7.8	14.8	9.6	10.7	11.6	—			
17	F.	53	100	45.4	Oral	Mild pellagra	0.55	0.75	0.51	0.60	3.9	4.4	7.4	5.9	4.4	12.7	6.7	5.5	8.3	6.4	9.9	8.9	8.3	11.2	10.1			
18	F.	47	125	56.8	Sub-cutaneous	Chronic pellagra with recent relapses	0.07	0.90	0.34	0.44	5.4	4.7	2.7	6.3	4.3	8.3	9.0	8.1	11.2	10.6	22.6	14.9	7.0	9.0	7.3			
19	F.	45	86	39.0	"	"	0.39	0.0	0.35	0.25	7.7	2.8	4.6	4.3	3.1	8.7	7.9	9.9	5.3	9.6	6.1	7.0	—	—				
Pellagrins; average:							0.33	0.55	0.40	0.45	5.7	4.0	4.9	5.5	3.9	9.9	7.9	7.8	7.6	10.0	12.5	10.0	9.2	—	—			
							*Urinary NM in mg. — pre-dosing NM average in mg. × mol.W. of NA × 100																					
							dosed NA in mg.																					

*Urinary NM in mg. — pre-dosing NM average in mg. × $\frac{\text{mol.W. of NA}}{\text{mol.W. of NM}} \times 100$
dosed NA in mg.

TABLE IV.—Response of Physically Fit Persons and Pellagrins to Subcutaneous Application of 500 mg. of NA Before, During, and After Daily Oral Dosage of 2 g. of Methionine (July 10 to 25, 1945)

No.	Sex	Age	Weight		Clinical Diagnosis	NM Elimination in mg. on Pre-dosing Days					Recovery of NA as (NM) in % of Ingested NA *										NM Elimination in mg. on Post-dosing Day			
						On 500 mg. NA Dosing Days					On 500 mg. NA-2 g. Methionine Dosing Days					On 500 mg. NA Dosing Days								
						1	2	3	Aver.	1	2	3	4	5	Aver.	1	2	3	Aver.	1		2	3	Aver.
20	F.	44	158	71.8	Physically fit	4.74	4.36	3.83	4.31	7.8	16.6	22.2	16.9	13.5	22.2	26.4	20.7	23.1	21.5	18.7	26.0	23.8	16.2	
21	F.	56	186	84.5	"	7.25	6.02	5.45	6.24	6.7	18.1	20.6	22.4	17.6	28.5	49.6	31.8	36.6	25.5	31.6	23.8	24.2		
22	F.	23	139	63.2	"	4.60	4.48	4.16	4.41	8.9	19.1	25.1	19.5	21.4	18.4	32.8	26.0	20.8	26.4	29.8	20.1	21.7		
23	F.	23	166	75.5	"	5.52	4.85	4.89	5.09	6.1	21.6	21.0	21.3	23.5	18.7	37.6	25.7	20.8	24.6	17.8	21.2	20.4		
24	F.	34	89	40.5	Malnutrition	4.78	4.20	5.54	4.83	6.0	20.3	24.7	25.4	27.2	20.7	37.6	41.9	39.6	29.6	14.4	27.2	20.3	28.4	
Normal controls; average:						5.38	4.78	4.77	4.98	7.1	19.1	22.7	21.1	20.6	18.1	31.7	33.9	26.7	30.8	23.2	19.1	25.4	24.2	22.2
25	F.	67	85	38.6	Acute pellagra	1.29	1.66	1.33	1.31	7.4	18.2	14.1	17.4	14.8	14.4	22.6	26.5	27.2	25.4	23.9	16.0	23.1	35.7	
26	F.	44	67½	30.7	Chronic	1.12	1.32	1.07	1.26	5.2	18.0	18.8	19.1	20.3	15.0	30.8	19.1	12.3	20.1	11.0	23.1	21.4		
27	F.	43	122½	55.7	Recurrence of pellagra—mild	1.48	1.25	1.04	1.26	3.4	9.9	16.1	16.8	15.3	12.3	41.9	25.6	18.5	28.7	15.2	14.0	16.6	13.3	
Pellagrins; average:						1.63	1.46	1.35	1.47	5.3	15.4	16.3	17.1	16.8	14.1	31.8	23.1	19.3	24.7	16.7	15.0	16.7	24.1	

* See note under Table III.

* See note under Table III.

TABLE V.—Recovery from Urine of NA (Eliminated as NM) after Dosing with 100 mg. of NA by Various Routes (Oct. 28 to Dec. 4, 1945)

No.	Sex	Age	Weight		Condition of Health	24 hrs. NM Elimination Pre-dosing Average in mg.	Recovered NA (as NM) in % of Dosed NA *						24 hrs. NM Elimination Post-dosing Average in mg.	
			lb.	kg.			During a 24-hr. Period after Dosing			During a 72-hr. Period after Dosing				
							Subcutaneous	Oral	Rectal	Subcutaneous	Oral	Rectal		
28	F.	32	128½	58.4	Fit (Wassermann +)	8.09	8.38	8.26	8.38	8.26	8.38	8.26	8.38	7.67
29	M.	40	164	74.5	Fit	5.96	8.46	9.40	8.46	9.40	8.46	9.40	8.46	5.67
30	F.	23	138	62.8	"	4.82	7.49	6.50	7.39	6.50	8.74	8.60	7.39	4.10
31	M.	31	153	70.0	"	3.50	8.80	9.30	7.49	7.68	10.52	12.32	7.81	4.17
32	M.	34	129	58.7	"	2.46	12.62	9.79	9.82	(7.26; 9.79)	16.94	10.16	12.04	3.74
33	F.	23	164	74.5	"	2.34	19.58	7.50	9.82	8.53	24.83	10.30	(6.66; 18.71)	5.90
34	F.	54	103	46.8	Mild pellagra	1.79	4.33	0.40	4.27	5.82	5.68; 5.46	5.82	5.26	2.37
35	F.	34	84	38.2	Malnourished and mild pellagra	1.31	11.15	4.66	3.21	4.27	15.89	8.39	(5.60; 6.78)	3.98
36	F.	55	113	51.4	Mild recurrent pellagra. Low food intake	0.62	7.14	(2.16; 4.82)	2.44	3.49	8.95	(2.16; 6.12)	2.87	1.28

* See note under Table III.

† A control rectal dosage with 100 mg. of NA revealed 1.49% recovery in 24 and 72 hours. The figures in black indicate the average of the results of two separate experiments carried out as first and last dosing experiments on each subject. The subjects are in order of their average pre-dosing NM elimination.

which had had a low nicotinamide elimination *in vivo*, and had low methylating efficiency *in vitro*. No systematic investigation of the effect of methionine on the urinary nicotinamide elimination by man has so far been carried out.

The limited number of experiments on physically fit persons and one diabetic provide identical results (Table I). No significant change of the daily nicotinamide methochloride output could be observed during a four-day period of oral intake of 2 g. of methionine. No significant quantitative alteration was seen in the response to 100 mg. of nicotinamide; this in spite of the fact that the second nicotinamide ingestion was made on the fourth day of methionine feeding. This was done to avoid a nicotinamide test during the refractory period, which was observed after methionine intake in some of the other experiments. Unfortunately no pellagrins suited to the experiment were available. The results are, however, so homogeneous that one can conclude that in physically fit people methionine is not a limiting factor for the height of the daily urinary elimination of nicotinamide methochloride or for the response to single doses of 100 mg. nicotinamide.

Experiments 2, 3, and 4, described in Tables II, III, and IV, were designed to exhaust, if possible, the body stores of "methyl-donators" by protracted ingestion of nicotinamide. The results vary individually and in the same person at different times to a considerable extent. In the March experiment (Table II) the ingestion of methionine depressed the response very considerably in No. 11 and moderately in No. 7 and for the first two days in Nos. 8, 9, and 10. In the latter three a considerable rise of the response occurred on the third day of methionine intake, which continued for the rest of the nicotinamide intake after discontinuing methionine; on the first of these days the output of No. 10 was even higher than the intake, which suggests the storage of nicotinamide. In Nos. 7 and 11 an increase occurred only on the second day after the end of methionine feeding.

In the May experiment (Table III) the results on the physically fit people were similar: the extent of the increase was somewhat less high than in the March experiment. In 3 pellagrins (Nos. 17, 18, and 19), however, a moderate rise of the response started immediately when methionine intake began and continued over the whole period of further nicotinamide intake. The response of the person who had had attacks of pellagra in recent years (No. 16) was intermediate between the fit persons and the pellagrins. It could be expected that an exhaustion of the body from "methyl-donators" would be more pronounced after the daily intake over a longer period of 500 mg. instead of 100 mg. of nicotinamide.

In 5 physically fit persons (Table IV) the intake of methionine caused already on the first day a considerable increase of the response, which continued to a lesser degree after discontinuing the methionine. The pellagrins, particularly No. 27, increased their response to nicotinamide considerably, and differences between pellagrins and non-pellagrins disappeared almost completely. This might be due to the pellagra-curing effect of a dosage 500 mg. of nicotinamide for 5 days before the first methionine intake.

To summarize: from Experiments 2, 3, and 4 (Tables II to IV) it is evident that methionine increases the response to ingested nicotinamide if relatively large doses of nicotinamide are ingested for a longer period. The effect is far more pronounced after the daily intake of 500 mg. than after that of 100 mg. In a nicotinamide-saturation test such as that recommended by Ellinger, Benesch, and Hardwick (1945) with a daily intake for a period of 5 days of 100 mg. of nicotinamide, the height of the nicotinamide metho-

chloride output might already have been affected by an exhaustion of "methyl-donators."

The most surprising result was the observation of a latent period in the action of methionine of up to 4 days in Nos. 7 and 11. An explanation of this phenomenon cannot be given. The oppression of the response to nicotinamide during the first days of methionine dosage suggests perhaps a blocking of the methylating mechanism of the liver.

The development of a nicotinamide deficiency is caused, apart from increased consumption effected by excessive exercise, etc., by insufficient nicotinamide intake from the diet or from the release of the intestinal flora. This can be due to malabsorption or to insufficient production or increased consumption or destruction of nicotinamide by the intestinal flora. Experiment 5 was designed to elucidate this point and to obtain more evidence about the response to nicotinamide when administered by various routes. From the investigation of Ellinger and Coulson (1944) it was known that orally ingested nicotinamide appeared in its methylated form in the urine of physically fit persons a few minutes after its ingestion; that after the intake of 100 mg. the elimination peak was reached in about 2 hours; and that the elimination was practically finished after 15 hours. This showed that methylation and absorption were very quick processes and that probably a considerable proportion of the orally ingested nicotinamide had been absorbed from the stomach. Consequently it could be expected that orally and parenterally ingested nicotinamide should give a similar response. It was, however, known from the paper by Ellinger and Coulson (1944) that orally ingested nicotinamide methochloride could be recovered only to a small extent—12–16%—while 62% was recovered after parenteral administration; findings confirmed by Perlzweig and Huff (1946). It was further known that in pellagrins but not in healthy persons the response to nicotinamide is frequently lower after oral than after parenteral application (Ellinger, Benesch, and Kay, 1945). Nothing was known about the fate of nicotinamide given per rectum. Prescott (1945) even doubts, without any evidence, that nicotinamide can be absorbed from the colon. The results shown in Table V provide a great variety of facts which have to be discussed.

1. In most of the 9 cases considered the elimination of the ingested nicotinamide in its methylated form is not finished 24 hours after the ingestion. The difference between 24-hour and 72-hour recovery after parenteral dosage is insignificant or absent on 4, slight on 3, and considerable on 5 occasions; after oral intake the difference is insignificant or absent on 3, slight on 3, and considerable on 6 occasions; after rectal intake the difference is insignificant or absent on 3, slight on 6, and considerable on 3 occasions. Only the considerable differences will be taken into account. If, as is the case with Nos. 32, 33, and 35, a considerable proportion of the recovered nicotinamide methochloride is eliminated later than 24 hours after ingestion independently of the route of application, one has to conclude that this is caused by a common factor other than the uptake from the intestines and either that the methylation or the elimination is slow or that part of the ingested nicotinamide is stored temporarily in the body. If, however, a large difference between the 24-hour and the 72-hour recovery is limited to the nicotinamide intake by one route, the mouth (cases Nos. 30, 31, and particularly 34), one has to conclude an interference with the nicotinamide uptake from the intestines. There was no large isolated nicotinamide methochloride elimination after the first 24 hours following rectal application.

2. Total response to parenteral and oral application was almost identical in 4 fit persons (Nos. 28, 29, 30, and 31), and in 1 pellagrin (No. 34). In 2 fit persons (Nos. 32 and 33) and 2 pellagrins (Nos. 35 and 36) nicotinamide methochloride elimination was far higher after parenteral than after oral ingestion; this indicates an interference with the dosed nicotinamide during its passage through the intestines. It might

be due to malabsorption: this is, however, improbable, since none of these cases showed a delayed elimination after oral ingestion alone. It is more probable that part of the ingested nicotinamide is either consumed or destroyed by the intestinal flora; this assumption is supported by the fact that in all these 4 cases the response to rectal is also much lower than to parenteral administration. In 2 cases (Nos. 29 and 31) the response to oral was somewhat higher than to parenteral dosage. This might have been due either to individual fluctuation or to an effect of the previous dosage with nicotinamide during the experiment.

3. In order to find out whether the repeated intake of nicotinamide altered the nicotinamide state of the body and therefore the extent of the response, at the end of each experiment a test was made with nicotinamide dosage by the route used at the beginning of the experiment. Considerable rises in response, such as in Nos. 32, 33, and 36, indicate an improved nicotinamide state in consequence of the repeated nicotinamide ingestions; this is confirmed by the considerably increased daily spontaneous elimination of nicotinamide methochloride in these 3 cases at the end compared with that at the beginning of the experiment. This factor has to be considered when the intermediate responses are valued.

4. In all cases a response was observed to rectally ingested nicotinamide. There can therefore be no doubt about the absorption of nicotinamide from the lower intestines. In most cases (Nos. 28, 30, 34, and 35) the response was somewhat but not much lower than that to oral ingestion. In 2 cases (Nos. 32 and 33) it was higher than that to oral dosage, and in both these cases there was a considerable rise of the nicotinamide state and response; and this explains the fact. In 3 cases (Nos. 31, 36, and particularly 29) the response to rectally ingested was considerably lower than that to orally ingested nicotinamide. This indicates bacterial consumption or destruction, since the whole ingested dose comes in contact with the intestinal flora when rectally applied, while most of the orally ingested nicotinamide is probably absorbed before it reaches the lower intestine and its flora.

Unfortunately it was impossible to obtain for the test more than three cases of pellagra, and all three were very mild cases. Nevertheless they gave a considerably lower response to orally ingested nicotinamide after 24 hours—3.28 mg.—as well as after 72 hours—5.35 mg.—than the physically fit persons—8.18 and 9.96 mg. respectively; and that is the only route which counts for the utilization of dietary and bacterial nicotinamide. From comparing the various values one can conclude that in Case 34, with very much delayed absorption and low response, the nicotinamide state is very low owing to malabsorption. In Case 35, with a delayed elimination after application by all three routes, the methylating mechanism might be faulty and the bacterial supply low on account of the low pre-dosing values and the considerable rise during the experiment. In Case 36, with its low oral response and small delay in elimination, pellagra might be caused mainly by insufficient bacterial supply. In Cases 32 and 33 the difference in response to parenteral and oral intake is considerable and the intestinal destruction of nicotinamide is great; the release of nicotinamide in the intestines must, however, be even higher than the destruction shown by the very high response to the parenteral application. Cases 28 to 31 show no peculiarities except perhaps for the low response of Case 29 to rectally applied nicotinamide. This, however, does not interfere with the normal nicotinamide uptake.

It is still impossible to obtain a complete balance of ingested nicotinamide in man. Small amounts are eliminated in the urine as such, more (between 2.87 and 24.83%) in our last experiment as nicotinamide methochloride. The rest cannot be accounted for. Of parenterally ingested nicotinamide methochloride only about 60% can be recovered. Perlzweig and Huff (1946) and Perlzweig, Huff, and Rosen (1946) believe that part of the nicotinamide methochloride is split up in the liver to unknown products,

and Najjar and Deal (1946) believe in a partial demethylation of nicotinamide methochloride in the liver. Both suggestions are improbable, since Ellinger (1947) could show that rat-liver slices do not destroy nicotinamide methochloride *in vitro*. It is more probable that a portion of the nicotinamide methochloride formed in the liver is eliminated with the bile into the intestine and there destroyed by the intestinal flora like the substance ingested by mouth.* This point cannot be elucidated by our method, but evidence can be obtained of a number of other factors concerned with state, uptake, and utilization of nicotinamide, and this might help to find the cause of a nicotinamide deficiency that might be different in each individual case.

The experiments show that the saturation test as described by Ellinger, Benesch, and Hardwick might be affected by an exhaustion of the "methyl-donators" of the body and therefore not provide a true picture of the nicotinamide state. It might be further affected by factors of the intestinal tract not connected with the nicotinamide state. Parenteral instead of oral application of the test doses would avoid the latter error.

A more satisfactory test, providing more detailed information about a variety of factors concerned with the state and utilization of nicotinamide, can be obtained by the following method: 24-hour urine samples are collected and the urinary nicotinamide methochloride is estimated for 18 days; immediately after the 3rd, 6th, 9th, and 12th collections 100 mg. of nicotinamide is administered—subcutaneously on the first and last, orally on the second, and rectally on the third occasion. The recovery percentage of the ingested nicotinamide is calculated for a 24-hour and a 72-hour period and the values obtained are analysed as in these experiments.

Summary

Evidence has been provided that lack of "methyl-donators" may affect the results of a nicotinamide-saturation test.

A new nicotinamide-saturation test has been described which avoids this error and supplies separate information about the nicotinamide state and the various factors involved in uptake from the gut and utilization of nicotinamide.

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* Cf. footnote page 672.