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Effect of Nicotinic Acid on Anoxia in Rats.

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On theoretical grounds, it is readily conceivable that the administration of nicotinic acid or its amide might be helpful, at least temporarily, in offsetting certain of the effects of reduced oxygen tension. As the prosthetic group of diphosphopyridine nucleotide, it has the capacity of accepting hydrogen from 3-phosphoglyceraldehyde, a triose resulting from the enzymatic breakdown of glucose. This transfer results in the release of energy at the expense of reduction of the coenzyme. Under ordinary aerobic conditions, the phosphoglyceric acid so produced undergoes enzymatic rearrangements to form pyruvic acid (von Euler *et al.*,[1]) which is then oxidized by way of thiamine and the flavoprotein-cytochrome systems to carbon dioxide and water. At the same time, reduced coenzyme is reoxidized and, thus reactivated, may again participate in the primary oxidation of triose. (Reviewed by Ball[2]).

Under anaerobic conditions, on the other hand, the failure of reduced coenzyme to be reactivated results in reorientations of the oxidative pattern, varying apparently on the degree of reduction of oxygen tension. Ball states[2] that if the oxygen tension is so reduced that the energy demands of the cell per unit of time are not met, then pyruvic acid and reduced pyridine nucleotide interact to yield lactic acid and oxidized coenzyme, this process finally being halted either by equilibrium conditions or acid formation. Kempner[3] has likewise shown that, as oxygen tension is reduced below a certain critical level, cellular respiration declines in rate and also changes qualitatively. Barron[4] is of the opinion that under such circumstances the release of energy depends on dehydrogenation rather than decarboxylation. These several lines of evidence are at variance with the earlier contentions of Meyerhof[5] and Warburg[6] that cellular respiration is entirely independent of oxygen tension and that it proceeds at a normal rate so long as the smallest amount of oxygen is present.

As corollary to the evidence just cited, it would appear that the enzymatic phases of cellular respiration might constitute a protective mechanism, useful in tiding the animal organism over a temporary period of reduced oxygen tension. This indeed may well be the device by which the tissues are enabled to accumulate an "oxygen debt" during excessive muscular exercise. Obviously, the effectiveness of this mechanism depends on an adequate supply of oxidized coenzyme; and if part of the latter be trapped in the reduced state because of lack of oxygen, then it is possible that the administration of nicotinic acid might augment the available supply of coenzyme and thus, in effect, correct this induced deficiency. Credence in this hy-

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Laboratory facilities were generously provided by the Department of Physiology and Pharmacology, School of Medicine, Duke University.

1 von Euler, H., Adler, E., Günther, G., and Hellström, H., *Z. physiol. Chem.*, 1937, **245**, 217.

2 Ball, E. G., *Bull. Johns Hopkins Hosp.*, 1939, **65**, 253.

3 Kempner, W., *Cellular and Comp. Physiol.*, 1937, **10**, 339; *Cold Spring Harbor Symposia on Quantitative Biology*, 1939, **7**, 269.

4 Barron, in discussion of Kempner (3-b).

5 Gildemeister, M., and others, editors, *Monographien aus dem Gesamtgebiet der Physiologie der Pflanzen und der Tiere*, Herausgegeben von M. Gildemeister, R. Goldschmidt, C. Neuberg, J. Parnas, und W. Ruhland. Band XXII: Die chemischen Vorgänge in Muskel und ihr Zusammenhang mit Arbeitsleistung und Wärmebildung, von Otto Meyerhof. Berlin, 1930, Julius Springer.

6 Warburg, O., and Kubowitz, F., *Biochem. Z.*, 1929, **214**, 4.

TABLE I.
Effect of Nicotinic Acid (or Its Amide) on Capacity of Rats to Survive at Low Barometric Pressures.

Pair No.	Untreated		Treated with nicotinic acid	
	Pressure at death, mm Hg.	Corresponding altitude, feet	Pressure at death, mm Hg.	Corresponding altitude, feet
1	80	52,000	60	58,000
2	80	52,000	60	58,000
3	100	47,000	60	58,000
4	80	52,000	40	66,000
5	80	52,000	80	52,000
6	80	52,000	60	58,000
7	80	52,000	60	58,000
8	80	52,000	60	58,000
9	100	47,000	80	52,000
10	80	52,000	80	52,000
11	100	47,000	100	47,000
12	100	47,000	100	47,000
13	100	47,000	80	52,000
14	80	52,000	60	58,000
15	100	47,000	100	47,000
16	100	47,000	100	47,000
17	200	32,500	80	52,000
18	80	52,000	80	52,000
19	80	52,000	80	52,000
20	80	52,000	80	52,000
Avg	93	49,275	75	53,800

pothesis is increased by observations of Kohn[7] and of Axelrod *et al.*[8] that the giving of nicotinic acid is followed by an increase in the concentration of the pyridine nucleotides. Decharneux[9] showed that the diethylamide of nicotinic acid (coramine) offsets some of the effects of high altitude (Cheyne-Stokes respiration, convulsions, and eventual death); and Morell,[10] noting an increase in the capacity for hard muscular exercise at sea level produced by nicotinamide, suggested that it might be beneficial in aviation.

In view of these theoretical considerations, it seemed worth while to investigate the effect of nicotinic acid on animals subjected to low barometric pressures. The results indicate that the tachypnea associated with reduced oxygen tension is significantly alleviated and that animals so treated are probably enabled to survive equivalent altitudes that otherwise would be fatal.

Experimental. (a) *Effect of Nicotinic Acid (and its Amide) on Capacity of Rats to Survive at Increasingly Low Barometric Pressures.* Twenty pairs of rats of the Vanderbilt strain[11] were used, their weights averaging 125 g. Two pairs at a time were placed in a desiccator and the air evacuated at a rate to simulate an ascent of 750 feet per minute. This rate was chosen so as to avoid the cumulative effects of gradually induced anoxia. Ventilation was assured by means of a small intake valve, and the room in which the experiments were conducted was air-conditioned (T 72° F; relative humidity, 50%). Half of the treated group received 1 mg nicotinic acid intraperitoneally,

[7] Kohn, H. I., *Biochem. J.*, 1938, **32**, 2075.

[8] Axelrod, A. E., Gordon, E. S., and Elvehjem, C. A., *Am. J. Med. Sc.*, 1940, **199**, 697.

[9] Decharneux, G., *C. R. Soc. de Biol.*, 1933, **112**, 692.

[10] Morell, T., *Deut. med. Wchnschr.*, 1940, **66**, 398.

[11] Wolfe, J. M., Bryan, W. R., and Wright, A. W., *Am. J. Cancer*, 1938, **34**, 352.

TABLE II.
Effect of Nicotinic Acid (1 mg) on Respiratory Rate of Rats at Barometric Pressure of 300 mm Hg. (Group A).

Animal No.	Respiratory rate at 760 mm	Respiratory rate at 300 mm	Absolute increase in rate
Untreated:			
1	72	120	48
2	80	174	94
3	108	192	84
4	90	192	102
5	120	216	94
6	108	186	76
7	96	162	66
8	132	252	120
9	96	150	54
10	120	192	72
Avg	102.2	183.6	81 (79.3%)
Treated with Nicotinic Acid:			
1	96	108	12
2	102	126	24
3	174	240	66
4	96	132	36
5	90	108	18
6	120	156	36
7	108	144	36
8	120	132	12
9	132	108	(—) 24
10	120	156	36
Avg	115.8	141	25.2 (21.8%)

Statistical analysis: Fisher's t test, on absolute increases in respiratory rates, gives a t value of 5.4572; degrees of freedom, 18; P is less than 0.001. The results therefore are highly significant.

and the other half an equivalent amount of nicotinamide; the control animals were injected with identical volumes of physiologic saline solution. The last gasp of each animal was accepted as a criterion of death.

Results of this experiment are shown in Table I. Since the behavior of the animals receiving free nicotinic acid and those treated with nicotinamide was identical, the two groups have been pooled in this table. Respiration of the untreated animals ceased at an average pressure of 93 mm Hg (corresponding to an altitude of 49,275 feet), whereas the final respiratory movements of the treated animals occurred at an average pressure of 75 mm Hg (53,800 feet elevation).

Statistical analysis by Fisher's t test yields a t value of 2.5245; degrees of freedom, 38; P then lies between 0.01 and 0.02. The results are therefore probably significant. It will be noted that one of the untreated animals died at a pressure of 200 mm Hg, while one of the treated animals survived until a pressure of 40 mm was reached. While the erratic behavior of these 2 animals unduly affects the average lethal pressures in the 2 groups, it does not, of course, affect the validity of the statistical conclusion.

(b) *Effect of Nicotinic Acid on Respiratory Rate of Rats at Low Barometric Pressures.* The animals in Group A, comprising 20 rats whose weights averaged 150 g, were subjected to a barometric pressure of 300 mm Hg attained gradually over a period of 30 minutes. In order to prevent violent fluctuations in pressure, a 5 gallon bottle was interposed between the vacuum pump and the desiccator holding the animals. Fresh air was admitted through a needle valve. Half of the animals were treated with 1 mg nicotinic acid in 0.5 cc diluent, and the other half received similar injections of physiologic saline solution. Respiratory excursions were recorded photographically by means of a differential pressure manometer designed by

TABLE III.
Effect of Nicotinic Acid Amide (5 mg) on Respiratory Rates of Rats at Barometric Pressures of 300 mm and 250 mm Hg. (Group B).

Animal No.	Respiratory rate at 760 mm	Respiratory rate at 300 mm	Increase	Respiratory rate at 250 mm	Increase
Controls:					
1	76	168	92	180	104
2	82	158	76	*	*
3	76	190	114	190	114
4	70	138	68	170	100
5	84	148	64	164	80
6	76	152	76	142	66
7	86	174	88	178	92
8	76	135	59	†	†
9	80	*	*	*	*
10	82	160	78	†	†
Avg	78.8	157.2	79.5 (100%)	172.3	93 (118%)
Treated:					
1	82	138	56	140	58
2	72	128	56	143	71
3	70	120	50	126	56
4	72	98	26	106	34
5	80	120	40	128	48
6	90	147	57	140	50
7	82	130	48	166	84
8	78	138	60	158	80
9	82	128	46	123	46
10	84	132	48	114	30
Avg	79.2	127.9	48.7 (61.5%)	134.9	55.7 (70.3%)

* Respiration failing; too irregular to be counted.
† Respiration ceased.
Statistical analysis: (1) Increases in rate at 300 mm Hg.: t, 4.8026; degrees of freedom, 17; P, less than 0.001. (2) At 250 mm Hg.: t, 4.015; degrees of freedom, 14; P, between 0.01 and 0.001. The differences in both sets of data are highly significant.

Hurst,[12] readings being taken before injection (at 760 mm), 10 minutes after injection (760 mm), and 40 minutes after injection (at a pressure of 300 mm Hg). In order to minimize psychic variants, all animals were trained by being placed in the apparatus daily for a week preceding the actual experiment.

The procedure in Group B differed from the above only in the following particulars: the animals averaged 225 g in weight; 5 mg nicotinamide was used instead of 1 mg nicotinic acid as in the first group; respirations were counted visually, as timed with a stopwatch; and observations were made both at 300 mm Hg and again at 250 mm.

Results of the experiment on Group A are shown in Table II. Since the rates before injection and 10 minutes afterward did not differ significantly, the latter have been omitted from the table. The absolute increase in respiratory rates of the untreated animals was 81 per minute (79.3%), while that of the treated animals was 25.2 per minute (21.8%). Fisher's t test on the absolute increases in rate shows that the results are highly significant.

Data on the experiment with Group B are shown in Table III. At 300 mm pressure, the average increase in respiratory rate of the untreated animals was 79.5 (100.0%), against an average increase of 48.7 (61.5%) in the animals receiving nicotinamide; the corresponding increases at 250 mm were 93 (118.0%) and 55.7 (70.3%) respectively. As will be noted from the table, 2 of the untreated animals died at 250 mm pressure,

12 Hurst, W., *Rev. Sci. Instruments*, 1941, **12**, 265.

and in several instances, both at 300 mm and at 250 mm, the respiratory movements of the untreated rats were too irregular to be counted with accuracy. This latter irregularity consisted of periodic breathing of the Cheyne-Stokes type, the periods of complete apnea being very long. The differences in respiratory rates of the contrasting groups, both at 300 mm and at 250 mm, are highly significant when analyzed by Fisher's t test.

Summary. Rats treated with nicotinic acid or its amide were found to survive until they reached a barometric pressure of 75 mm Hg (equivalent altitude, 53,800 feet); untreated controls died at an average pressure of 93 mm Hg (49,275 feet). Statistically, the differences are probably significant.

The tachypnea developing at low barometric pressure is much less in treated than in untreated rats. Preliminary injection of 1 mg nicotinic acid was followed by an increase in respiratory rate of only 22% at 300 mm Hg as opposed to approximately 80% in untreated controls. Differences of comparable magnitude were noted at pressures of 300 and 250 mm Hg following the administration of nicotinamide. Statistical analyses indicate that these differences are highly significant.

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Effect of 3,3'-Methylene-Bis-4-Hydroxycoumarin (Dicumarol) on Embryonic Chick Liver and Spleen.*

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Dicumarol is being used today in the control and possible prevention of thrombo-embolic phenomena. Its mode of action is as yet not well understood. Some workers have presumed that it works by inhibiting the utilization of vitamin K by the liver cell in the synthesis of prothrombin. Others think that it may act as a toxic agent on the liver cells, especially when used in large amounts.[1] This study was initiated to observe the effect of dicumarol on isolated sections of liver and spleen of the 14 day chick embryo.

Method. 14 day chick embryos were removed from their shells and placed in petri dishes containing Tyrode's solution. The liver and spleen were isolated and small portions were placed in individual petri dishes containing various concentrations of dicumarol in a 10% rabbit serum-Tyrode solution. Since the volume of the medium used was small, the pieces of tissue were sectioned in the petri dishes containing the dicumarol in the given concentrations. This was done in order to prevent dilution of the dicumarol serum-Tyrode medium. These pieces of liver and spleen were then cut into approximately one cubic millimeter sections with a sharp scalpel and placed on 20 x 20 mm No. 2 cover slips in a small drop of media containing the disodium salt of dicumarol in 10% rabbit serum and Tyrode's solution. These cultures were placed on deep-welled micro culture slides in a lying-drop position, sealed with paraffin and incubated at 38° C. The disodium salt was prepared by adding the crystalline material to .345 N sodium hydroxide. The same amount of base was added to the media containing the different amounts of dicumarol. The pH of the media ranged from 7.74 to 7.88. Little or no proliferation of cells was noted at 24 hours. This was especially true in the liver cultures. Therefore, the period of observation was begun at 48 hours. The longest period of observation was 72 hours. According to Nordman, before 14 days an extensive proliferation of

* This study was supported by a grant-in-aid from the United States Public Health Service.

1 Rose, C. L., Harris, P. N., and Chen, K. K., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 228.