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Effect of Dinitrophenol on Experimental Diabetes.

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(Introduced by Louis Leiter.)
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The metabolic action of 2-4-dinitrophenol has been the subject of several recent and informative studies. Thus the greatly increased oxygen consumption characteristic of its administration to animals and man seems from the observations of Hall, Tainter, Cutting and coworkers[1, 2] to be largely at the expense of fat as a fuel, at least when the stimulus augmenting metabolism is not too abrupt and overwhelming. There is general agreement that dinitrophenol does not increase the output of nitrogen in the urine[1-5] and hence protein catabolism seems to be little affected. The injection of large and generally fatal doses into animals causes some elevation of the blood sugar and a considerable loss of liver and muscle glycogen, but smaller doses have little effect on the blood sugar.[2, 6, 7] The glycemic effect of large doses of dinitrophenol is not dependent upon asphyxia[8] and examination of the blood shows no very significant shift in the acid-base balance.[2, 8, 9] Studies of the ammonia, organic acid and ketone body excretion of patients administered dinitrophenol indicate that the increased oxidation of fat is complete.[1, 3, 4] In fact, as Tainter, Cutting, and Hines[1] have stated the salient feature in the study of dinitrophenol is the negative findings in so many phases of its metabolic action.

The administration of a different oxidative stimulant, thyroid extract, to partially depancreatized dogs results in a severe and even

[1] Tainter, M. L., Cutting, W. C., and Hines, E., *J. Pharm. and Exp. Therap.*, 1935, **55**, 326.

[2] Hall, V. E., Field, J., Sahyun, M., Cutting, W. C., and Tainter, M. L., *Am. J. Physiol.*, 1933, **106**, 432.

[3] Cutting, W. C., and Tainter, M. L., *J. A. M. A.*, 1933, **101**, 2099.

[4] Dunlop, D. M., *Brit. Med. J.*, 1934, **1**, 524.

[5] Furth, O., and Rapoport, S., *Biochem. Z.*, 1934, **272**, 81.

[6] Magne, H., Mayer, A., and Plantefol, L., *Ann. de Physiol. et de Physiochim. Biol.*, 1932, **8**, 51.

[7] Magne, H., Mayer, A., and Plantefol, L., *Ann. de Physiol. et de Physiochim. Biol.*, 1932, **8**, 71.

[8] Hall, V. E., Brown, C. A., and Sahyun, M., Proc. Soc. Exp. Biol. and Med., 1933, **31**, 380.

[9] Muntwyler, E., Myers, V. C., Danielson, W. H., and Zorn, C., *Am. J. Physiol.*, 1935, **113**, 186.

fatal diabetes,[10, 11] and similarly, thyroid medication, or the spontaneous occurrence of hyperthyroidism, greatly increases the severity of human diabetes, inhibiting the action of insulin and often precipitating ketosis.[12] In the past the generally accepted explanation of the adverse effect of hyperthyroidism on diabetes mellitus has been that advanced by Wilder,[12] namely that with the important exception of exercise, "measures or conditions which stimulate the general metabolism have a depressing action on the tolerance of the diabetic patient."

It appeared to us worthwhile to test this explanation by studying the effect on diabetes of the markedly increased metabolism resulting from the administration of dinitrophenol to the depancreatized dog maintained on insulin. If hypermetabolism *per se* increases the severity of diabetes an augmented glycosuria might be expected from this procedure, and the greatly increased oxidation of fat in the presence of a limited carbohydrate consumption, should, according to generally accepted ideas, favor the development of ketosis. The entirely negative outcome of the experiment about to be described, together with its lengthy and painstaking character discouraged us from extending our observations to more than the one dog. We believe, however, that the experiment is of sufficient interest to warrant a brief description, especially in view of 2 recent reports which lead to similar conclusions. Wishnofsky and his coworkers[13] observed that the administration of dinitrophenol to human diabetics did not increase their hyperglycemia either in the fasting state or after the ingestion of dextrose, and that the glycosuria was decreased by the drug. These experiments were of two hours' duration. Barker[14] recently reported that the excretion of ketone bodies by completely diabetic dogs was not augmented by the markedly increased oxidation of fat resulting from hypermetabolism induced by the administration of dinitrophenol. Apparently the adverse effect of spontaneous or experimental hyperthyroidism on diabetes is not due solely to the increased metabolism which accompanies that condition, and dinitrophenol produces no striking alteration of the metabolic abnormalities of diabetes mellitus.

The male dog used in this experiment was completely depancreatized in November, 1935, and the observations were made in May

[10] Allen, F. M., *J. Metabol. Res.*, 1922, **1**, 619.

[11] Shpiner, L. B., *Am. J. Physiol.*, 1930, **92**, 672.

[12] Wilder, R. M., *Arch. Int. Med.*, 1926, **38**, 736.

[13] Wishnofsky, M., Kane, A. P., Shlevin, E. L., and Byron, C. S., *Arch. Int. Med.*, 1935, **56**, 374.

[14] Barker, S. B., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 893.

of 1936. In January he was placed on a weighed diet of beef heart, sugar, raw pancreas, cod liver oil and salt mixture offered in 2 feedings with 15 units of insulin at each feeding and this regime remained unchanged throughout the observation. For the 3 months before the experiment his diabetic condition was quite constant and he excreted from 15 to 30 gm. of glucose a day. During this time his food was all consumed and his weight did not vary.

Metabolism experiments[15] were made in the fasting state in the morning before the administration of insulin or dinitrophenol. The expired air was collected in a spirometer and analyzed with a Haldane apparatus. Analyses were done in triplicate, and each respiratory quotient and total metabolism in Table I represents the average of 2 or 3 ten-minute periods. The dog was trained to lie quietly and the constancy of the oxygen consumptions and respiratory quotients is evidence of satisfactory technic. The urine was collected between catheterizations. Urinary sugar was determined by the Somogyi-Shaffer-Hartmann method applied to 1/100 dilutions of urine treated by the Somogyi zinc precipitation method. Experiments with yeast showed that in this dilution the non-fermentable reducing substances in the urine did not cause a significant error. Urine total nitrogen was determined by the macro Kjeldahl method,

TABLE I.
Effect of Dinitrophenol on a Depancreatized Dog Maintained on Insulin.

			Urine					
Date	Weight, Kg.	Dinitro-phenol, Mg.	Sugar, G/24H	Organic Acids, MEq/24H	Total N G/24H	Creat., G/24H	Total R.Q.	Basal Metabolism, Cals./H
5/13			27.4	35	8.6	.45	.70	21.8
5/14	11.7		41.1	44	9.6	.46	.68	23.5
5/15			36.8	40	9.3	.46	.68	21.9
5/16			18.6	34	8.8	.42		
5/17			19.9	50	8.9	.41		
5/18	11.7		34.3	44	9.9	.43	.66	22.6
5/19		/30	43.8	39	9.8	.40	.69	21.6
5/20		30/30	27.3	35	9.2	.39	.69	22.6
5/21	11.6	60/60	27.5	50	8.8	.42	.68	22.1
5/22	11.5	60/60	23.5	33	8.6	.41		
5/23	11.5	60/60	16.1	42	8.4	.45		
5/24	11.6	90/90	21.2	29	7.9	.43		
5/25	11.5	90/60*	17.2	39	8.9	.43	.70	23.3
5/26	11.6	120/120	17.1	30	7.0	.37	.72	21.2
5/27	11.7	120/60	17.9	80	8.7	.41	†	†
5/28	11.6	60/60/60	17.7	34	8.6	.41	†	†

*This dose and all subsequent were administered as the sodium salt of dinitrophenol.

†See Table II.

[15] Boothby, W. M., and Sandiford, I., *Laboratory Manual of the Technic of Basal Metabolic Rate Determinations*, Philadelphia, 1920.

TABLE II.
Effect of Dinitrophenol on Metabolism of a Depancreatized Dog.

Date	Time	Dinitrophenol, Mg.	Total R.Q.	Metabolism, Cals./H
5/27	8:15 A.M.	120		
	10:00		.73	45.1
	11:00		.73	59.7
	11:15 P.M.	60		
5/28	8:30 A.M.	60		
	10:24		.68	34.2
	10:55		.71	32.0
	11:55		.71	28.4

creatinine by the Folin method, and organic acids by the method of Van Slyke and Palmer. These procedures were all carried out as described by Peters and Van Slyke.[16]

The results are presented in Tables I and II. In Table I, column 3 gives the morning and afternoon dose of dinitrophenol which was administered in capsules with the food. On the afternoon of 5/25 and for the rest of the experiment we changed to the sodium salt of dinitrophenol administered the same way to see if the drug was more effective in that form. Calculation of the nitrogen balance, allowing for the fecal nitrogen 10% of the food nitrogen, shows that for the first 9 days the dog lost 1.1 gm. N a day, and for the last 7 days he was practically in balance (a loss of 0.2 gm. N per day).

It will be noted that up to the last 2 days the metabolism, determined in the morning, was not elevated. That this was because the effect of the evening dose of the drug had worn off by morning, about 15 hours later, is shown by Table II, in which on the last 2 days of the experiment the metabolism was determined at intervals *after* the administration of sodium dinitrophenol. Table II indicates that the doses used could cause a significant elevation of metabolism for a period of hours. The effect of the drug may have been prolonged to some extent by our habit of giving it with the food, but we were unable with 2 doses a day to produce a constant elevation such as occurs in the human, possibly because dinitrophenol is more rapidly destroyed and excreted by the dog. The negative character of the experiment in all other respects is shown in Table I.

Summary. The administration of dinitrophenol and its sodium salt was without effect on the fasting total respiratory quotient, urinary sugar, organic acids, total nitrogen and creatinine of a depancreatized dog maintained on a constant diet and with insulin.

[16] Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry, Vol. II, Methods,* Baltimore, 1932.

The metabolic effect of the drug, apparent for some hours after its administration, had always disappeared by the time the metabolism was determined in the morning about 15 hours after the last dose. The significance of these results is discussed earlier in the paper.

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Relationship Between Peptone Shock and Anaphylactic Shock.

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We have recently reported that the intravenous injection into dogs of a solution of peptone produces shock indirectly by leading to the liberation of a vasodepressor substance, identified as histamine, from the tissues.[1] Thus the mechanism of peptone shock is apparently similar to that of anaphylactic shock.[2] Biedl and Kraus[3] pointed out the remarkable similarities between these conditions. They also reported that peptone shock desensitizes an animal to anaphylactic shock and *vice versa*. They concluded that the fundamental mechanism concerned in these reactions was identical. They interpreted the reactions as indicating that peptone contains some active substance, such as Popielski's vasodilatin, which can produce shock directly, and that in the anaphylactic experiment a mother substance is formed as the result of the sensitization which then discharges this active peptone constituent when the shocking injection of antigen is made. Subsequent investigators have had variable results in attempting to influence an anaphylactic reaction by the prior administration of peptone. Hill and Martin[4] in their review summarize the reports as follows: "In short, there is some, but not striking evidence that peptone may inhibit shock within certain defined limits."

In view of our demonstration that both peptone shock and anaphylactic shock are reactions that are brought about indirectly by

[1] Dragstedt, C. A., and Mead, F. B., *J. Pharm. and Exp. Therap.*, 1937, **59**, 429.

[2] Dragstedt, C. A., and Mead, F. B., *J. Pharm. and Exp. Therap.*, 1936, **57**, 419.

[3] Biedl and Kraus, *Wien. Klin. Wchnschr.*, 1909, **22**, 363.

[4] Hill, J. H., and Martin, L., *Medicine*, 1932, **11**, 141.