THE INFLUENCE OF CARBOHYDRATE ON THE ORIGIN OF SYMPTOMS AND THE ONSET OF DEATH IN THIAMINE-DEFICIENT PIGEONS*

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INTRODUCTION

As has been known for some time, administration of larger amounts of carbohydrate —in most cases given in replacement of fat—to thiamine-free fed animals causes an earlier occurrence of the symptoms of deficiency and death[1,2]. We concluded from previous experiments that this effect was due to the increased depletion of the thiamine pyrophosphate (TPP) stores of the tissues of thiamine-free fed animals on administration of increasing amounts of carbohydrate[3] (in animal tissues vitamin B_1 is practically entirely present as TPP). As had been suggested by YUDKIN[4], the possibility remained, however, that a second effect of carbohydrate administration would exist besides this depleting action, *viz.* an influence on the level of TPP in the tissues at which symptoms and death would occur. For example, it might be the often presumed toxic effect of intermediates of carbohydrate metabolism upon the thiamine-deficient organism. In order to investigate the possible existence of such a second effect of carbohydrate an experiment was carried out in which the TPP content of several tissues was determined after death by thiamine deficiency. The TPP content at death was chosen in preference to that at the first occurrence of symptoms in order to avoid arbitrariness in the recognition of symptoms, and because former experiments had indicated that animals receiving different amounts of carbohydrate exhibit different pictures of symptoms of thiamine deficiency.

EXPERIMENTAL PART

Methods

The pigeons and the composition of the diets were the same as described in a previous paper[5].

Thiamine pyrophosphate (TPP) was determined by the manometric method. Care was taken that no more than four hours elapsed between death and preparation of extracts for TPP determination, in which time there is no loss of TPP from whole organs, as has already been stated[6]. In most cases the extracts were prepared immediately after death. In a number of tissues nitrogen determinations were also carried out, but there were no differences of any importance between the groups investigated.

* This work forms part of investigations on the metabolism and physiological function of thiamine carried out by H. G. K. WESTENBRINK and collaborators.

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For the statistical calculation of the P-value of the difference between the groups the method of WILCOXON[7] was used owing to the non-normal character of the distribution; for the calculation of "trend" the rank correlation method of KENDALL[8] or its modification for groups by TERPSTRA[9] were used.

Two groups of 45 pigeons each were given the following thiamine-free food each day: group A 2 g casein and 18 g sucrose, group B 2 g casein and 8 g refined soy bean oil. The food was supplemented with a salt and vitamin mixture[5]. It was administered by forced feeding in order to avoid the undesired influence of anorexia. The pigeons were weighed every third day and were watched day and night in order to observe character and severity of symptoms and to be able to examine them as soon as possible after death. 9 animals of group A died in the first week without having shown symptoms of thiamine deficiency, and 8 of group B. These pigeons were not analyzed. Upon post-mortem investigation most of them appeared to have died from other diseases. Pigeon 284 of carbohydrate-free group B was the only animal still alive after 73 days. It was then sacrificed and the TPP contents of its organs were determined. The results of all TPP determinations, together with other relevant data, are given in Table I, while Table II shows the mean TPP values of both groups and the P-value of difference. In order to investigate the influence of the time that elapsed between the beginning of the experiment and death on the weight loss and TPP content within groups the trend of these values against time was calculated by the methods mentioned. To demonstrate the order of magnitude of the differences involved the mean values of the TPP content were calculated for the first and second half of both groups of pigeons and are presented together with the level of significance of the trend in Table III. The weight loss in both groups was significantly different ($P < 0.001$) and showed a significantly increasing trend in group A ($P < 0.001$). At first sight it appears to have a declining trend for group B, but this tendency is mainly determined by the three pigeons of this group that died first. If their values are omitted, the trend is statistically insignificant ($P > 0.2$).

In accordance with former experiments the weight loss in group A must be ascribed to the frequent vomiting which is a consequence of thiamine deficiency[5]. The pigeons receiving oil usually did not vomit much.

The symptoms showed an interesting feature. While only 4 out of 36 pigeons of group A, the group receiving carbohydrate, had opisthotonus, 31 of the 36 pigeons of group B, receiving no carbohydrate, exhibited this symptom. In both groups animals had severe spasms, and shortly before death the behaviour seemed to indicate occurrence of cramps of the heart or respiratory system.

The time between appearance of the first symptoms (equilibrium disturbance) and death was often less than one day for the pigeons of group A, while a number of days, in some cases more than a week, elapsed between appearance of the first symptoms and death in the animals of group B.

In several cases these pigeons showed a spontaneous recovery from opisthotonus, and pigeons 216, 295 and 346 only exhibited symptoms again three to eight days after this recovery.

The TPP contents at death show a remarkable picture. While the animals of group A had a significantly lower content of liver and heart, as compared to those of group B, the contrary was the case for the contents of breast muscle and cerebrum. All these differences are highly significant. In all four organs of pigeons of group B the TPP

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TABLE

TIME OF DEATH, WEIGHT, CHANGE OF WEIGHT, TPP CONTENT AT (RECEIVED CARBOHYDRATE)

Pigeons were fed diets A and B respectively for the first time on 8/10. at = ataxia; eq = disturbance of equi-

GROUP A

Pigeon No.	*Time of death*	*Initial weight (g)*	*Δ weight (g)*	*μg TPP per g tissue*				*Symptoms in order of appearance*
				Liver	*Heart*	*Breast muscle*	*Cerebrum*	
345	19/10 23^{00}	349	— 18	0.82	1.52	3.10	1.49	lw, sp
202	20/10 0^{30}	410	— 85	1.87	0.69	2.82	1.56	vm, eq (s)
351	20/10 0^{30}	342	— 60	1.72	1.67	3.25	1.57	vm, eq (s)
333	20/10 9^{40}	332	— 7	1.57	1.30	3.52	1.84	lw, eq
357	21/10 5^{30}	370	— 49	1.03	1.18	2.93	1.70	eq, lw
318	21/10 15^{30}	378	— 66	0.75	1.05	3.08	1.65	eq (s)
230	21/10 20^{50}	379	—102	2.34	2.50	3.59	2.58	eq ++, sp
204	22/10 8^{30}	427	— 1	0.55	0.88	2.49	0.98	eq, sp
316	22/10 11^{30}	355	— 46	1.44	1.17	1.90	1.71	at, eq
352	22/10 11^{30}	449	— 51	0.96	1.21	2.20	0.86	eq
301	22/10 14^{50}	370	— 66	1.10	1.03	2.95	1.08	sp, eq, op
337	22/10 14^{50}	411	— 81	0.48	0.35	2.56	1.44	lw, eq
296	23/10 10^{00}	441	— 83	1.00	0.72	2.46	0.95	at, eq (s)
304	23/10 10^{00}	361	— 70	1.47	1.12	3.62	1.59	at, sp
314	23/10 16^{00}	373	— 87	1.92	1.68	2.58	2.08	eq
313	23/10 20^{00}	475	—112	0.62	1.07	2.90	0.70	at (s), op
954	23/10 20^{00}	404	— 85	0.37	0.60	2.22	1.33	eq, sp
210	23/10 23^{10}	390	— 37	0.67	0.60	3.24	—	sp
238	24/10 14^{00}	466	—139	0.69	0.39	1.92	1.40	at, op
298	24/10 17^{00}	441	—129	0.77	1.17	3.97	0.52	vm
359	24/10 17^{30}	397	— 92	0.57	0.56	3.24	0.87	eq (s)
322	24/10 20^{30}	372	— 55	0.92	0.39	2.10	0.74	eq, lw, sp ++
339	24/10 20^{30}	393	— 83	0.95	0.85	2.18	1.16	at, eq (s), sp
214	25/10 10^{30}	465	— 91	0.70	0.42	2.48	0.84	vm, lw, at
200	25/10 16^{00}	430	—127	—	—	—	—	lw
218	25/10 16^{00}	418	—105	0.82	0.50	3.72	0.56	eq, op
319	25/10 16^{40}	417	— 98	0.91	0.53	2.74	1.09	eq, sp
332	26/10 5^{00}	360	— 89	1.54	1.21	2.79	1.80	eq (s)
240	26/10 15^{20}	426	—122	1.03	1.09	3.60	0.68	sp ++, op
331	27/10 0^{10}	483	—167	1.13	0.98	2.82	1.20	eq (s)
294	27/10 16^{00}	474	—175	1.42	1.26	3.86	0.77	eq +, sp, op
282	27/10 16^{10}	393	— 91	0.81	0.84	1.66	0.92	eq, sp ++
340	27/10 22^{20}	391	— 96	1.24	1.49	2.75	1.40	eq +, lw
222	28/10 6^{00}	369	— 84	0.38	0.59	2.16	0.98	eq (s), lw
326	31/10 11^{00}	411	—104	1.45	1.49	2.36	1.52	eq (s), lw, sp ++
310	1/11 14^{10}	384	— 99	1.38	0.98	1.96	1.35	eq (s), lw, sp
mean		*403*	*— 85*	*1.08*	*1.00*	*2.79*	*1.26*	

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DEATH AND NATURE OF THE SYMPTOMS OF PIGEONS OF GROUP A AND B (NO CARBOHYDRATE)

librium; lw = leg weakness; op = opisthotonus; sp = spasms; vm = vomiting; (s) = slight; + = severe, etc

GROUP B

Pigeon No.	*Time of death*	*Initial weight (g)*	*Δ weight (g)*	*μg TPP per g tissue*				*Symptoms in order of appearance*
				Liver	*Heart*	*Breast muscle*	*Cerebrum*	
334	26/10 15^{15}	397	—121	1.60	3.50	2.66	2.81	eq +, vm
300	2/11 20^{30}	433	—125	2.42	1.89	2.84	2.24	eq (s)
323	6/11 11^{30}	367	—120	2.46	2.86	2.94	2.32	eq (s)
237	7/11 16^{00}	408	— 35	1.76	1.41	1.64	0.78	eq, sp ++, op ++
320	8/11 14^{00}	380	— 46	1.28	1.22	1.70	0.93	eq +, at, sp +, op +, lw
223	8/11 15^{00}	494	— 71	1.44	1.45	3.07	0.76	at, sp +, op ++
219	11/11 13^{00}	345	— 2	2.49	1.66	1.81	0.80	eq, sp +, op
324	11/11 23^{30}	339	+ 38	1.93	1.30	1.40	0.77	eq, sp +, op
201	12/11 15^{35}	402	+ 14	1.71	1.32	1.94	0.65	eq, op
358	12/11 16^{10}	400	— 67	1.60	1.27	2.06	0.60	eq, lw, op, sp
344	13/11 11^{00}	356	— 31	1.74	1.33	1.54	0.81	eq, lw
227	13/11 14^{15}	417	— 55	1.60	1.51	1.77	0.71	eq, op, sp
317	14/11 4^{50}	485	— 85	1.83	2.10	2.24	0.76	eq +, op, sp +
303	15/11 7^{00}	460	— 53	1.68	1.56	2.90	0.68	op, sp ++
354	16/11 18^{15}	425	— 12	1.80	1.69	2.60	0.94	op, sp
293	16/11 18^{20}	424	— 70	1.82	1.43	2.06	0.71	sp +, op
315	17/11 8^{00}	312	+ 3	2.09	1.17	1.53	0.86	eq, lw, sp +, op
297	18/11 1^{40}	458	— 76	1.22	0.97	2.08	0.99	eq, op, sp +
216	18/11 14^{00}	426	+ 13	1.29	1.02	1.65	0.25	eq, op, sp ++ (9/11)
336	19/11 15^{30}	409	— 50	0.87	0.87	1.11	0.55	op, sp + [eq ++, lw, sp
312	21/11 14^{20}	386	— 9	1.36	1.09	1.53	0.66	eq, op, sp +
329	21/11 20^{30}	375	— 6	1.73	1.32	1.59	0.74	eq, lw, op, sp
341	29/11 13^{50}	372	+ 17	1.77	0.87	1.47	0.51	eq, sp ++, op, lw
330	30/11 8^{20}	366	— 91	1.49	0.76	1.29	1.01	eq, lw, sp
221	30/11 22^{25}	466	— 48	1.26	1.22	1.60	0.48	eq, lw, sp ++, op
335	1/12 13^{40}	457	+ 21	1.66	1.52	1.73	0.42	lw, op ++, sp +
327	2/12 5^{30}	366	+ 1	1.59	1.85	1.75	0.81	eq, op, sp ++
343	3/12 16^{35}	394	— 92	1.62	1.25	1.45	0.48	eq +, op, lw, sp
239	6/12 17^{00}	314	+ 34	1.49	1.09	1.27	0.87	op, sp, lw
328	8/12 17^{35}	365	— 3	0.96	0.86	1.64	0.39	eq, lw, op, sp +
233	10/12 16^{00}	346	— 38	1.17	1.34	1.15	0.83	eq, vm, op
203	13/12 5^{00}	414	— 58	1.46	1.21	1.26	0.52	eq +, sp ++, op
346	13/12 11^{30}	392	— 14	1.12	1.22	0.96	0.59	eq, sp ++, op, lw
295	15/12 19^{30}	346	+ 12	0.96	0.97	0.72	0.29	eq, sp +, op +
309	15/12 20^{00}	379	+ 7	1.24	1.04	0.88	0.30	eq, sp +, lw, op +
325	17/12 0^{15}	395	— 20	1.40	0.60	0.88	0.37	eq, sp ++
284 killed	20/12 12^{30}	408	+ 12	1.87	1.04	0.79	1.09	eq (s)
mean without *284*		*396*	*— 34*	*1.58*	*1.38*	*1.74*	*0.81*	

content appeared to be lower as the period between withholding of thiamine and death became longer. In the animals of group A only the cerebrum showed this effect.

TABLE II

TPP CONTENTS (μg/g TISSUE) OF THE ORGANS OF PIGEONS DEAD FROM THIAMINE DEFICIENCY

Group A: received carbohydrate
Group B: received no carbohydrate

Organ	*Group A*		*Group B*		*P-value of difference*
	Number	*Mean*	*Number*	*Mean*	
liver	35	1.08	36	1.58	<0.001
heart	35	1.00	36	1.38	<0.001
breast muscle	35	2.79	36	1.74	<0.001
cerebrum	34	1.26	36	0.81	<0.001

TABLE III

DECLINING TREND OF TPP-VALUES AT DEATH AGAINST SURVIVAL TIME

Group A	*First half-period*	*Second half-period*	*P-value of trend*
Group A			
liver	1.15	1.02	0.42
heart	1.13	0.87	0.15
breast muscle	2.86	2.72	0.15
cerebrum	1.47	1.05	0.01
Group B			
liver	1.80	1.34	<0.01
heart	1.65	1.12	<0.01
breast muscle	2.15	1.33	<0.01
cerebrum	1.06	0.56	<0.01

DISCUSSION

The depleting effect of carbohydrate on the TPP content is different for different organs. Even after the very long time that elapses between the beginning of the experiment and death, pigeons fed a diet containing no thiamine and no carbohydrate do not attain the low TPP values in liver and heart at the moment of death that the animals receiving carbohydrate have, while the latter animals do not attain the low values at death in breast muscle and cerebrum that those have which receive no carbohydrate. It appears possible that, by administration of carbohydrate, certain organs lose so much of their TPP that survival becomes impossible, while on a carbohydrate-free diet the attainment of the lethal level is so much delayed that other organs reach the lethal TPP level sooner. We have investigated only four rather arbitrarily chosen organs, but other organs, parts of organs or cell fractions in certain organs might be concerned, which we will take together here under the term "vital centres". If the TPP level in these centres reaches a certain critical level, death occurs. The same applies for the symptoms; the figures indicate that opisthotonus might be related to a critical low level of TPP in some part of the brain, but it is also possible that in the animals

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of Group A opisthotonus only appeared for so short a time that it escaped detection.

If in both groups the same vital centre was responsible for death, we can assume that the influence of carbohydrate on the velocity of the decline of the TPP content in this centre would be intermediate between that on heart and liver, on the one hand, and breast muscle and brain on the other hand. So the TPP level in this centre would be decisive for death, independent of the quantity and composition of the thiamine-free food. We cannot entirely exclude the existence of a second effect of carbohydrate in this case, whereby in this centre a difference in lethal level would exist between pigeons fed carbohydrate and those receiving no carbohydrate. We believe, however, that at present there is no reason to assume the existence of such a second effect and that it is desirable to use a minimal number of hypotheses when trying to explain phenomena.

The fact that all the organs examined of the animals of group B and the cerebrum of animals of group A showed a lower TPP value as the time between the beginning of the experiment and death was longer points to the possibility of a kind of adaptation to the loss of the vitamin in certain animals, be it only short-termed.

Such an adaptation is known to occur in other cases. It might provide an explanation for YUDKIN's results[4], but we consider it more likely that his diet contained just enough thiamine for survival in the absence of carbohydrate. More experiments will be required to decide this point.

SUMMARY

Former experiments had shown that increased carbohydrate consumption leads to a quicker depletion of the thiamine pyrophosphate stores of pigeons on diets deprived of thiamine. Now thiamine pyrophosphate has been determined in liver, heart, breast muscle and cerebrum immediately after death by vitamin-B_1 deficiency. It appeared that on a carbohydrate-rich diet the content of liver and heart was lower, and the content of breast muscle and cerebrum was higher than on a diet in which carbohydrate was replaced by an isocaloric amount of fat. These results suggest that the rapid depletion of the stores of thiamine pyrophosphate is the only cause of the earlier appearance of symptoms and of the earlier death by introduction of a higher percentage of carbohydrate in the thiamine-deficient diet. There is no reason to assume any toxic effect of carbohydrate or its metabolic intermediates upon the thiamine-deficient organism.

RÉSUMÉ

Des expériences précédantes avaient montré qu'une augmentation de la consommation d'hydrate de carbone a pour effet une diminution accélérée des réserves de pyrophosphate de thiamine chez le pigeon recevant une diète exempte de thiamine. Actuellement, le pyrophosphate de thiamine a été déterminé dans le foie, le coeur, le muscle pectoral et le cerebrum immédiatement après la mort par déficience de vitamine B_1. L'auteur a constaté qu'avec une diète riche en hydrate de carbone la teneur du foie et du coeur était plus basse, celle du muscle pectoral et du cerveau plus élevée qu'avec une diète où l'hydrate de carbone était remplacé par une quantité isocalorique de matière grasse. D'après ces résultats il semble que la diminution rapide des réserves de pyrophosphate de thiamine soit la seule raison pour laquelle les symptomes apparaissent et la mort survient plus rapidement, lorsque le pourcentage d'hydrate de carbone dans une diète exempte de thiamine est augmenté. Il n'y a pas de raison de supposer que l'hydrate de carbone ou ses intermédiaires métaboliques exercent une action toxique quelconque sur l'organisme à carence de thiamine.

ZUSAMMENFASSUNG

Frühere Versuche hatten gezeigt, dass erhöhte Kohlenhydratzufuhr bei der Taube auf thiaminloser Diät eine raschere Verminderung der Thiaminpyrophosphatvorräte zur Folge hat. Das Thiaminpyrophosphat wurde nun in der Leber, im Herz, im Brustmuskel und im Grosshirn sofort nach dem

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Tode durch Vitamin B_1-Mangel bestimmt. Es hat sich gezeigt, dass bei kohlenhydratreicher Diät der Gehalt von Leber und Herz niedriger, von Brustmuskel und Gehirn aber höher war als bei einer Diät in der das Kohlenhydrat durch eine isokalorische Menge Fett ersetzt war. Diese Versuche weisen darauf hin, dass die rasche Abnahme der Thiaminpyrophosphat-Reserven die einzige Ursache sei, auf welche das verfrühte Auftreten der Symptome und des Todes bei Erhöhung des Kohlenhydratgehaltes in thiaminarmer Diät zurückzuführen ist. Es besteht kein Grund anzunehmen, dass das Kohlenhydrat oder seine metabolischen Zwischenprodukte eine toxische Wirkung auf den thiaminarmen Organismus ausüben.

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