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Summary

By the reaction of 2,6-lupetidine with phosphorus oxychloride a new phosphorylating agent, 2,6-lupetidylphorodichloridate was synthesized. Unprotected adenosine was phosphorylated with this reagent on 5′-hydroxyl group in a yield around 15%.

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Studies on the Metabolism of Borate. III.*1 Variations of Fructose 6-Phosphate Levels and Fructose 1,6-Diphosphate Levels in some Organs and Blood after Administration of Borate, and Effects of Boron on Anaerobic Glycolysis.

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During the studies on the biochemical actions of borate, the authors suspected that borate might inhibit the anaerobic glycolysis of glucose. For the purpose of studying the effects of boron upon the sugar metabolism, the authors examined influences of boron upon the levels of fructose 6-phosphate (F6P) and fructose 1,6-diphosphate (FDP) in some organs and blood of the rats and guinea pigs, and also investigated effects of boron upon the anaerobic glycolysis.

It was found that the animals received borate orally in a single dose of 140 mg. or 240 mg./kg. apparently showed a tendency to rise the FDP levels in livers within 4 hours, while they showed no significant change of the F6P levels in livers as well as levels of both F6P and FDP in brains, kidneys and blood. It was also found that the anaerobic glycolysis of glucose in homogenates of livers and brains of the animals was considerably inhibited by borate, and the rate of the inhibition was higher in the former.

Methods and Results

Animals, Diet and Dosage——Rats (0.200~0.250 kg., body wt.) and guinea pigs (0.370~0.500 kg., body wt.) were used. The keeping, diets and the method of administration of borate were the same as the previous report.*4

Determination of F6P and FDP in Biological Materials——F6P and FDP in biological materials were determined spectrophotometrically by the method described by Roe, *et al.*[1]

*1 Part II. M. Akagi, T. Misawa, H. Kaneshima : Yakugaku Zasshi, **83**, 209 (1963).
*2 Nishi-5-chome, Kita-12-jo, Sapporo (赤木満洲雄).
*3 Nishi-15-chome, Minami-2-jo, Sapporo (三沢隆行, 金島弘恭).
*4 Part I. M. Akagi, T. Misawa, H. Kaneshima : Yakugaku Zasshi, **82**, 934 (1962).

1) J.H. Roe, *et al.* : J. Biol. Chem., **210**, 703 (1954).

Measurement of Anaerobic Glycolysis——The assay of anaerobic glycolysis was carried out by the method of LePage[2] except the use of Thunberg tube and lactic acid produced was determined by the method of Barker-Summerson.[3]

Effects of Boron upon F6P Levels and FDP Levels——Concentrations of F6P and FDP in some tissues such as livers, brains, kidneys, and blood of the rats and guinea pigs were determined 4 hours after oral administration of borax in a single dose of 140 mg. and 240 mg./kg. in term of boron to a rat and to a guinea pig respectively. Table I shows the levels of F6P and FDP in the organs and blood of the animals treated and control animals. In brains, kidneys and blood there appeared to show no change of levels of the both phosphates, while a considerable rise in the levels of FDP and no considerable change of the levels of F6P were found in the livers. Fig. 1 represents the FDP levels in livers of both animals treated and control animals.

Table I. F6P Levels and FDP Levels before and after Administration of Borate

		Livers			Brains			Kidneys			Blood		
Sex	Body wt. (kg.)	F6P	FDP (μg./g.)	Total	F6P	FDP (μg./g.)	Total	F6P	FDP (μg./g.)	Total	F6P	FDP (μg./g.)	Total
						Rats							
Control													
♂	0.250	180	224	332	10	48	58	23	60	83	7	68	75
♂	0.240	80	210	290	—	—	—	—	—	—	—	—	—
♂	0.230	91	304	395	—	—	—	—	—	—	—	—	—
♂	0.200	63	290	353	0	5	5	5	95	100	0	146	146
	Average		257±23[a]	343±22									
Test													
♂	0.250	66	699	765	25	49	74	36	82	121	3	18	21
♂	0.230	10	682	692	13	14	27	35	99	134	0	99	99
♂	0.200	40	614	654	2	26	28	23	36	59	6	54	60
♂	0.250	59	857	916	10	38	48	11	45	56	0	21	21
	Average		713±51[b]	757±57[b]									
						Guinea Pigs							
Control													
♂	0.450	7	320	327	29	43	72	17	68	85	15	36	51
♂	0.500	30	350	380	—	—	—	—	—	—	—	—	—
♂	0.420	24	274	298	—	—	—	—	—	—	—	—	—
♂	0.370	47	304	351	16	89	105	4	57	61	7	17	24
	Average		312±16	339±17									
Test													
♂	0.450	59	598	657	20	37	57	10	74	84	14	42	56
♂	0.500	85	559	644	30	44	74	25	115	140	14	35	49
♂	0.450	23	472	495	10	59	69	42	118	160	10	31	41
	Average		543±37[b]	599±52[b]									

The levels were determined at 4 hr. after oral administration of borate in a dose of 240 mg./kg. to a rat and 140 mg./kg. to a guinea pig as boron.

a) Plus or minus values represent the standard error of the mean.

b) Significantly different from controls ($p=0.01$).

Effects of Boron upon Anaerobic Glycolysis——With 10% liver and brain homogenates of the rats, the tests of anaerobic glycolysis of glucose, by the measurement of lactic acid after 30 minutes incubation was carried out after addition of 0.04*M* boric acid-potassium bicarbonate buffer to the reaction mixture (0.01*M*, final concentration). Table II shows that anaerobic glycolysis was inhibited significantly by boron and the average inhibition

2) G.A. LePage: J. Biol Chem., **178**, 100ç (1948).

3) S.B. Barker, W.H. Summerson: *Ibid.*, **138**, 535 (1941).

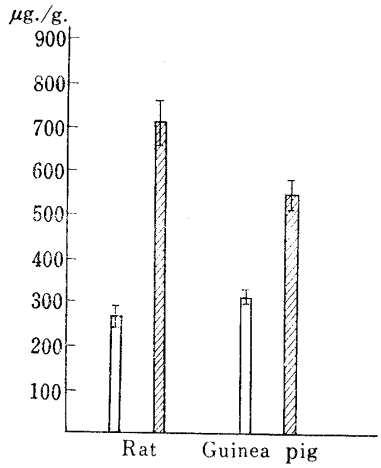

Fig. 1. Comparison of FDP Levels in the Livers of Animals Received Borate with that of the Control Animals

□ : Control (4 rats, 4 guinea pigs)

▨ : Levels after 4 hr. (240 mg./kg. to 4 rats and 140 mg./kg. of boron to 3 guinea pigs)

The vertical line gives the standard error of the mean.

Table II. The Results of Inhibition of Anaerobic Glycolysis by Boron

	Amount of lactic acid produced per ml. in react. mixt. No boron (μg.)	Boron added (μg.)	Inhibition rate (%)
Rat liver homogenate	38	8	79
	42	16	62
	47	30	36
	17	14	18
	8	4	50
	10	6	40
	12	4	66
	50	18	64
	36	8	77
		Average	$55\pm6.8^{a)}$
Rat brain homogenate	58	40	31
	86	63	27
	78	44	44
	94	74	21
	96	80	17
	88	68	23
	54	38	30
		Average	$28\pm3.3^{a)}$

Reaction mixture : the same as the LePage's one. In the tests boric acid-potassium bicarbonate buffer solution (pH 7.6) was substituted for H_2O and its final concentration was 0.01*M*. Reaction vessel : Thunberg tube. Temp.: 38° Time 30 min. pH : 7.6. Reagent for removing protein : 10% trichloroacetic acid.

a) Plus or minus values represent the standard error of the mean.

rates were 55 ± 6.8 (S. E) % and 28 ± 3.3 (S. E) % in rat liver and brain respectively.

Discussion

Winfield[4] described that phosphorylase which participates in the metabolism of starch to G-1-P was accelerated by 0.1*M* borate, whereas the reverse reaction was inhibited, and Zittle,[5] *et al.* reported that alkaline phosphatase which takes part in the reaction of G-6-P to glucose, was also inhibited by boron. In our unpublished experiments, hexokinase which participates in the reverse reaction of glucose to G-6-P was found not

4) M.E. Winfield : Aust. J. of Exper. Biol. & Med. Science, **23**, 269 (1945).
5) C.A. Zittle, *et al.* : Arch. of Biochem., **26**, 112 (1950).

to be influenced by boron. From these facts it was suspected that the accumulation of FDP in liver should be explained by either the accelerations of enzyme reaction in G6P or/and F6P to FDP, which occurred in the steps before FDP, or the inhibitions of enzyme system in the course from FDP to lactic acid. Since in our experiments, however, the anaerobic glycolysis was found to be inhibited evidently by boron, the accumulations of FDP in livers treated with boron might be caused by the disturbance of sugar metabolism by the inhibition in the anaerobic glycolysis.

It is necessary to investigate which of the enzymes in anaerobic glycolysis is inhibited by boron, but a fall of ATP-production caused by the facts noted above may also be accounted for one of the important factors of boron poisoning.

The authors wish to thank Dr. Y. Nakamura, the head of this Institute of Public Health, for his giving the chance of study.

Summary

Both the levels of fructose-6-phosphate (F6P) and fructose 1,6-diphosphate (FDP) in biological materials such as livers, brains, kidneys and blood of rats and guinea pigs were investigated at 4 hours after oral administration of borax in a single dose of 140 mg. and 240 mg./kg. as boron to the former animals and to the latter respectively, and boron effects upon anaerobic glycolysis were also examined with liver homogenates and brain homogenates of rats.

FDP levels in livers of these animals received borax had a tendency to rise obviously within 4 hours.

Anaerobic glycolysis of glucose was found to be inhibited considerably by boron (0.01 *M* boric acid), being the inhibition rates higher with liver homogenates than with brain homogenates of rats.

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