THE CONVERSION OF ANEURIN INTO ANEURINPYROPHOSPHATE BY BLOOD CORPUSCLES*

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INTRODUCTION

Not only the amount of aneurin ingested with the diet, but also the absorption of aneurin from the intestine and its conversion into aneurinpyrophosphate (APP) by the body cells are important factors conditioning the vitamin B_1 content of the body. The absorption has been studied by FRIEDEMANN *et al*[1]. The problem of the conversion into APP formed the background of the investigations described in this paper.

WILLIAMS AND BISSELL[2] studied the *in vivo* conversion of aneurin into APP and found that a few minutes after the injection of 15 mg aneurin into the vena of one arm the APP content of the blood taken from the other arm was increased two to three times. In experiments on blood *in vitro* WESTENBRINK AND STEYN-PARVÉ[3] had found that in contrast to the blood of some other species human blood, prevented from clotting by Na oxalate, did not synthesize APP from added aneurin. This contradiction prompted us to repeat the experiments of WILLIAMS AND BISSELL[2]. We could not detect a significant increase of the APP content of the blood after the injection of 25 mg aneurin (in one experiment the APP content was 9.0 γ per 100 ml before and 9.3 γ three minutes after the beginning of the injection; in a second experiment the corresponding figures were 9.8 and 9.3). But moreover in an *in vitro* experiment similar to those of WESTENBRINK AND STEYN-PARVÉ, with the only difference that heparin was used as an anticoagulant instead of oxalate, we observed that human blood had a definite, although low capacity of APP synthesis. This observation suggested that oxalate might inhibit the synthesis of APP and has been the starting point of our further experiments.

EXPERIMENTAL PART

Aneurinpyrophosphate formation by human blood and rat blood treated with various anticoagulants

Human blood and rat blood respectively were prevented from clotting by 0.3%

* This work forms part of the investigations on aneurin metabolism by H. G. K. WESTENBRINK and collaborators.

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Na oxalate, 0.05% heparin ("Vitrum") or 0.2% Na citrate. Other samples were examined after defibrination. Before and after incubation with 1 mg aneurin per ml at 37° C (human blood) or 39° C (rat blood) the APP content was estimated by means of the method of WESTENBRINK *et al*[4]. The results are assembled in Tables I and II. These results show that oxalate inhibits the formation of APP from added aneurin. This inhibition is not caused by the removal of Ca ions as these are also removed by citrate.

TABLE I

FORMATION OF APP BY *human blood* TREATED WITH VARIOUS ANTI COAGULANTS OR DEFIBRINATED

Blood sample	Anti-coagulant	γ APP per 100 ml after incubation with 1 mg aneurin per ml at 37° C for		
		0 h	½ h	1 h
1	Oxalate	9.8	10.6	10.5
	Heparin	9.8	11.2	14.6
2	Oxalate	8.0	—	11.2
	Heparin	8.0	—	16.6
	Defibrinated	8.0	—	15.9

TABLE II

FORMATION OF APP BY *rat blood* TREATED WITH VARIOUS ANTI-COAGULANTS

Blood sample	Anti-coagulant	γ APP per 100 ml after incubation at 39° C for						
		0 h	1¾ h	2 h	4 h	6 h	8 h	24 h
1	Oxalate	19.8	—	102	137	—	—	25
2	Heparin	19.2	—	285	440	555	—	111
3	Citrate	22	179	—	436	454	415	—

Influence of enzyme inhibitors on the formation of aneurinpyrophosphate by rat blood

Oxalate is known as an inhibitor of glycolysis. We also studied the influence of other enzyme inhibitors, *viz.*, monoiodoacetate, fluoride and cyanide on the synthesis of APP from added aneurin by the cells of rat blood. The results are assembled in Table III.

These results suggest that processes yielding energy as glycolysis and respiration are necessary for the synthesis of APP. More evidence could be obtained by studying the influence of oxalate and cyanide on the synthesis of APP by separate red and white blood cells. The red cells mainly cover their energy requirements by glycolysis, while in white cells respiration is the main source of energy (see p. 538).

The mixed blood of various rats, prevented from clotting by 0.05% heparin, was divided into 4 parts of 10 ml each. The substances indicated in Table IVA were added,

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TABLE III

INFLUENCE OF MONOIODOACETATE (M.I.A.), FLUORIDE AND CYANIDE ON THE SYNTHESIS OF APP FROM ADDED ANEURIN (1 mg per ml) BY RAT BLOOD

	Additions	γ APP per 100 ml after incubation at 39° C for						
		0 h	$1^3/_4$ h	$2^3/_4$ h	4 h	5 h	6 h	8 h
Total blood	0.2% Na citrate	22	179	—	436	—	454	415
	0.2% Na citrate + 0.01 *m* cyanide	22	133	—	154	—	121	113
Erythrocyte suspension	—	15.2	—	286	—	470	—	—
	$^1/_{320}$ *m* Na fluoride	15.2	—	200	—	257	—	—
	$^1/_{160}$ *m* Na fluoride	15.2	—	132	—	195	—	—
	$^1/_{80}$ *m* Na fluoride	15.2	—	42	—	(35)	—	—
	$^1/_{4500}$ *m* M.I.A.	15.2	—	282	—	447	—	—
	$^1/_{1500}$ *m* M.I.A.	15.2	—	200	—	403	—	—
	$^1/_{500}$ *m* M.I.A.	15.2	—	155	—	228	—	—

after which the blood samples were incubated for 3 hours at 39° C and the APP contents of the red and white blood cells were determined by the method described in an earlier paper[5]. It appeared to be necessary to centrifuge the blood for a longer time than blood which had not been incubated with aneurin in order to achieve a sufficient separation of the red and white cells. The results are assembled in Table IVB.

The following conclusions can be drawn:-

1. Leucocytes have a much greater synthesizing capacity for APP than erythrocytes. This holds even when the increase of the APP content is expressed in % of the amount of APP initially present, while a normal rat leucocyte already contains about 160 times as much APP as a normal red cell.

2. The synthesis of APP by the leucocytes is more strongly inhibited by cyanide than by oxalate.

3. The synthesis of APP by the erythrocytes is more strongly inhibited by oxalate than by cyanide.

In order to compare these conclusions with the catabolic activities of the blood cells, we must calculate the energetic effect of glycolysis and respiration of the red and white cells in the presence and in the absence of inhibitors. The extent of glycolysis and respiration is usually expressed as μl CO_2 liberated from bicarbonate buffer by the lactic acid formed, respectively μl O_2 taken up by 1 mg cell protein (dry weight) per hour (Q_G and Q_{O_2}). We can express the energy yield as the amount of high energy phosphate bonds (ATP) formed by these processes. In glycolysis 1 mol. CO_2 is aequivalent to 1 mol. ATP, in respiration 1 mol. O_2 is aequivalent to 6 mol. ATP, assuming that the P/O ratio is 3. Thus the ratio of the ATP-yields of respiration and glycolysis is about six times the ratio $\frac{Q_{O_2}}{Q_G}$. As reliable data concerning the Q-values for rat erythrocytes were not

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TABLE IV

INFLUENCE OF OXALATE AND CYANIDE ON THE SYNTHESIS OF APP BY RED AND WHITE CELLS OF RAT BLOOD

A. SUBSTANCES ADDED TO 10 ml RAT BLOOD

Addition	I	II	III	IV
Aneurin: 10 mg	—	+	+	+
Na oxalate: 30 mg (in 1st exp. 50 mg)	—	—	+	+
Cyanide: 0.14 ml of a solution of 170 mg NaCN per 5 ml + 0.36 ml 0.28 *n* HCl; final concentration 0.01 *m* HCN	—	—	—	+

B. APP CONTENTS OF THE BLOOD CELLS AFTER 3 HOURS' INCUBATION AT 39° C (Q = RATIO OF APP CONTENTS OF A WHITE AND A RED CELL)

		γ APP per 10^{11} cells		Q
		Erythrocytes	Leucocytes	
I. Untreated	1st experiment	1.9	285	147
	2nd experiment	2.5	500	200
	3rd experiment	2.7	490	185
II. Incubated with aneurin	1st experiment	34.4	18800	550
	2nd experiment	48.5	26600	550
	3rd experiment	46.7	25300	540
III. Incubated with aneurin + oxalate	1st experiment	—	—	—
	2nd experiment	8.0	12000	1500
	3rd experiment	4.3	16300	3800
IV. Incubated with aneurin + cyanide	1st experiment	18.5	7700	420
	2nd experiment	31.0	8700	280
	3rd experiment	32.5	12300	380

available to us, we used the data for human erythrocytes, in which the ratio of glycolysis and respiration may be presumed to be about the same as for rat erythrocytes. The following figures are given in literature.

Human erythrocytes:
- $Q_{O_2} = 0.025$ (HARROP AND BARRON[6], and DAMBLE[7])
- $Q_G^{N_2} = 0.25$ (BIRD[8] (anaerobic glycolysis))
- $Q_G^{O_2} = 0.22$ (BIRD[8] (aerobic glycolysis))

Rat leucocytes:
- $Q_{O_2} = 9$ (FUJITA[9])
- $Q_G^{N_2} = 20$ (FUJITA[9] (anaerobic glycolysis))
- $Q_G^{O_2} = 2.6$ (FUJITA[9] (aerobic glycolysis))

Presuming, firstly that cyanide completely inhibits respiration and oxalate glycolysis, and secondly that inhibition of either respiration or glycolysis does not interfere with the other process, we have calculated the ATP production under different conditions

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(as in connection with the problem discussed only the ratios have to be considered, the units in which the amounts of ATP should be expressed have been omitted):-

erythrocytes:	$6 \times 0.025 + 0.22 = 0.37$
erythrocytes + oxalate:	$6 \times 0.025 + 0 = 0.15$
erythrocytes + cyanide:	$0 + 0.25 = 0.25$
leucocytes:	$6 \times 9 + 2.6 = 56.6$
leucocytes + oxalate:	$6 \times 9 + 0 = 54$
leucocytes + cyanide:	$0 + 20 = 20$

Thus the ATP production from the catabolism of the erythrocytes decreases by addition of oxalate to about 40% and by addition of cyanide to about 70% of the initial value and the ATP production of the leucocytes decreases by addition of oxalate to about 95% and by addition of cyanide to about 35% of the initial value.

As the presumptions mentioned above are not completely admissable, these figures can only be considered as rough approximations. Nevertheless the following conclusions seem to be justified:-

1. Leucocytes have a much larger production of ATP than erythrocytes.

2. The production of ATP by the leucocytes is much more inhibited by cyanide than by oxalate.

3. The production of ATP by the erythrocytes is much more inhibited by oxalate than by cyanide.

When we compare these conclusions concerning the energy producing processes in the red and white blood cells with our conclusions concerning the capacity of these cells to synthesize APP we may conclude that *glycolysis and respiration are the main sources of the energy necessary for the synthesis of APP from aneurin in erythrocytes and leucocytes respectively.*

We have tried to stimulate the production of ATP by the addition of methylene blue or toluidene blue to the blood. These dyes increase the respiration, and as this would possibly cause an increase in the ATP production, an investigation of the influence of these dyes on the APP formation seemed to be a valuable method to control the assumption that ATP is the link between systems producing energy and the phosphorylation of aneurin.

However, we did not observe any stimulatory effect on the formation of APP, but depending upon the concentrations of the dyes used, a more or less inhibitory effect on the normal synthetic activity of the blood cells. This quite agrees with the view of LOOMIS AND LIPMANN[10] according to which "dyes as methylene blue and cresyl blue, although accelerating respiration, disrupt the link to phosphorylation and replace inorganic phosphate".

Influence of the concentration of aneurin on the synthesis of aneurinpyrophosphate by blood corpuscles

In the experiments described above very high aneurin concentrations were always used, *viz.*, 1 mg per ml of blood. We also determined the amount of APP formed at lower aneurin concentrations. The results are summarized in Table V.

Obviously the synthesis of APP at the higher levels of aneurin does not differ essentially from that at lower levels. Indeed with smaller amounts of aneurin added

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TABLE V

INFLUENCE OF THE ANEURIN CONCENTRATION ON THE SYNTHESIS OF APP BY RAT BLOOD TREATED WITH HEPARIN

Aneurin γ per ml	γ APP per 100 ml after incubation for		% phosphorylated after 4½ hours
	4½ hours	6¼ hours	
1000	393	—	0.25
500	311	331	0.39
200	255	252	0.78
100	246	221	1.5
50	200	173	2.4
20	145	126	4.1
0	23.2	23.6	—

Aneurin γ per ml	γ APP per 100 ml after incubation for		% phosphorylated after 4 hours
	2 hours	4 hours	
20	135	183	5.4
10	108	126	7.1
3	—	74	12
1	38.0	36.6	12
0.3	25.6	26.4	17
0.1	22.1	22.8	26
0	19.0	18.9	—

a higher percentage is converted into APP, but even with the smallest amount studied no complete conversion was obtained. While of 1 mg added to 1 ml blood about 0.25% is phosphorylated in about 4 hours, 26% was phosphorylated of 0.1 γ.

Influence of the time of incubation on the amount of aneurinpyrophosphate synthesized

From the experiments described above it appears that at first the synthesis of APP from added aneurin proceeds fairly rapidly. The APP concentration then remains practically constant for some hours and later on it declines (see, *e.g.*, Table II). Various explanations present themselves for this course of the APP content of the blood on incubation with aneurin:-

1. The decrease of the reaction velocity might be explained by breakdown of the added aneurin or by inhibition of the synthesis by reaction products. These possibilities could be excluded, however, by the following experiment, in which we used a low aneurin concentration (1 γ per ml blood) in order to obtain the greatest possible influence

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of aneurin breakdown. Because the amount of APP formed with this aneurin concentration is fairly small we used the mixed blood of rats having lived for 4 weeks on an aneurin free diet. Blood of these animals has a very low APP content, so that small increases can be accurately determined. 10 ml of this blood prevented from clotting by 0.05% heparin was incubated for 2 ½ hours with 1 γ of aneurin per ml at 39° C (sample I). Thereupon 8 ml were centrifuged and the plasma obtained was added to the cells obtained from a sample of 8 ml of the same mixed blood which had been stored at 0° C. To another sample of the stored blood 1 γ aneurin per ml was added. Both these samples were incubated for 2 ½ hours at 39° C. We then found the following APP concentrations:-

a. Blood stored at 0° C: 3.4 γ per 100 ml,

b. Blood stored at 0° C and incubated for 2 ½ hours with 1 γ of aneurin per ml at 39° C: 22.5 γ per 100 ml,

c. Plasma of sample I mixed with the cells of blood stored at 0° C; the mixture incubated for 2 ½ hours at 39° C: 22.0 γ per 100 ml of mixture.

Obviously no destruction of aneurin had occurred and no inhibiting degradation products had been formed.

2. Free APP cannot exist in cells as it is rapidly destroyed by the phosphatase. Therefore we presume that it is bound to protein immediately after its formation. So the slowing down of the accumulation of APP in the blood cells might be caused by lack of bearer protein. The next experiment demonstrates that this cannot be the only cause.

The blood of 4 rats, prevented from clotting by 0.05% heparin, was mixed and divided into 6 equal portions. Portions I to V were placed in the incubator at 39° C, portion VI was stored at 0° C. After 0, 3, 6, 9 and 26 hours respectively 1 mg aneurin per ml was added to portions I to V. This amount of aneurin was also added to portion VI after 26 hours' storage at 0° C. The samples were then incubated at 39° C. APP was determined after 0, 3, 6 etc. hours. The results are assembled in Table VI.

TABLE VI

INFLUENCE OF STORING AT 0° C AND INCUBATION AT 39° C WITHOUT ADDED ANEURIN ON THE CAPACITY OF RAT BLOOD FOR SYNTHESIZING APP (AMOUNT OF ANEURIN ADDED: 1 mg per ml)

Treatment before the addition of aneurin	γ APP per 100 ml after incubation with aneurin for					
	0 h	3 h	6 h	9 h	12 h	15 h
I. Fresh	15.4	341	332	249	249	232
II. 3 h at 39°	15.0	42	60	75	—	—
III. 6 h at 39°	14.3	25	33	38	—	—
IV. 9 h at 39°	13.7	33	36	—	—	23
V. 26 h at 39°	10.4	21	32	—	—	—
VI. 26 h at 0° C	15.8	260	270	—	—	—

The figures of Table VI show that the capacity for synthesis of APP has considerably decreased after 3 hours' incubation at 39° C in the absence of aneurin. So lack of bearer protein cannot be the only cause of the decrease of the reaction velocity.

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3. The concentration of the substrates necessary for energy production decreases during incubation. As glucose breakdown is the main source of energy production in these cells the influence of the glucose concentration on APP formation was investigated.

We determined the amount of APP formed from added aneurin (1 mg or 3 γ per ml) by:

a. Rat erythrocytes in a medium which approaches physiological conditions, *viz.* rat plasma from which glucose had been removed by treatment with yeast;

b. Rat erythrocytes and human erythrocytes washed with Ringer solution and thereupon suspended in this solution;

c. Total rat blood and total human blood;

d. Rat erythrocytes and human erythrocytes suspended in untreated plasma.

TABLE

INFLUENCE OF THE CONCENTRATION OF GLUCOSE (IN THE MEDIUM) ON THE SYNTHESIS OF APP FROM ADDED

No.	Subject	Medium	Aneurin added	Glucose (mg per 100 ml)
1.	Rat erythrocytes	Plasma	1 mg/ml	0
	Rat erythrocytes	Plasma	1 mg/ml	20
	Rat erythrocytes	Plasma	1 mg/ml	100
2.	Rat erythrocytes	Ringer sol.	1 mg/ml	0
	Rat erythrocytes	Ringer sol.	1 mg/ml	60
	Rat erythrocytes	Ringer sol.	1 mg/ml	120
3.	Rat erythrocytes	Ringer sol.	3 γ/ml	0
	Rat erythrocytes	Ringer sol.	3 γ/ml	60
	Rat erythrocytes	Ringer sol.	3 γ/ml	120
4.	Total human blood	—	1 mg/ml	100
	Total human blood	—	1 mg/ml	250
	Total human blood	—	1 mg/ml	450
	Total human blood	—	1 mg/ml	650
5.	Total human blood	—	1 mg/ml	100
	Human erythrocytes	Plasma (untreated)	1 mg/ml	100
	Human erythrocytes	Plasma (untreated)	1 mg/ml	2300
	Human erythrocytes	Ringer sol.	1 mg/ml	0
	Human erythrocytes	Ringer sol.	1 mg/ml	100
	Human erythrocytes	Ringer sol.	1 mg/ml	2300
6.	Total rat blood	—	1 mg/ml	280
	Rat erythrocytes	Plasma (untreated)	1 mg/ml	280
	Rat erythrocytes	Plasma (untreated)	1 mg/ml	2300
	Rat erythrocytes	Ringer sol.	1 mg/ml	130
	Rat erythrocytes	Ringer sol.	1 mg/ml	280
	Rat erythrocytes	Ringer sol.	1 mg/ml	2300
7.	Rat erythrocytes	Ringer sol.	1 mg/ml	0
	Rat erythrocytes	Ringer sol.	1 mg/ml	150
	Rat erythrocytes	Ringer sol.	1 mg/ml	300
	Rat erythrocytes	Ringer sol.	1 mg/ml	600

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Various amounts of glucose were added. The results are assembled in Table VII.

These figures show that red cells suspended in glucose free plasma or in Ringer solution without added glucose have a much smaller synthesizing capacity than the same cells in a medium containing about 100 mg % glucose. In the first hours of incubation the absence of glucose in the medium only has a slight depressing effect. Obviously the cells themselves contain a sufficient reserve of substrate for sustaining a level of metabolism adequate for normal synthesis of APP for some hours.

Raising the concentration of glucose from 100 mg % to 300 or 600 mg % does not enhance the synthesis of APP. In the presence of increased glucose concentrations the cells obviously do not use more glucose than in the presence of the physiological concentration (about 100 mg %). Higher concentrations of glucose (*e.g.*, 2300 mg %) even

VII

ANEURIN BY TOTAL HUMAN AND RAT BLOOD AND BY ERYTHROCYTES SUSPENDED IN DIFFERENT MEDIA

	γ APP per 100 ml after incubation at 39° C for										
0 h	1 h	2 h	2½ h	3 h	4 h	4½ h	5 h	6 h	7 h	8 h	10 h
17	—	—	200	—	—	194	—	—	—	—	—
17	—	—	214	—	—	228	—	—	—	—	—
17	—	—	218	—	—	307	—	—	—	—	—
20	—	127	—	—	—	—	—	152	—	141	—
20	—	140	—	—	220	—	—	380	—	323	—
20	—	140	—	—	272	—	—	465	—	575	—
18	—	—	49	—	—	—	45	—	—	—	—
18	—	—	59	—	—	—	74	—	—	—	—
18	—	—	69	—	—	—	94	—	—	—	—
8.3	—	13.9	—	—	16.6	—	—	21.2	—	21.5	—
8.3	—	12.5	—	—	16.1	—	—	20.0	—	21.4	—
8.3	—	12.1	—	—	16.3	—	—	20.4	—	21.3	—
8.3	—	13.2	—	—	16.9	—	—	20.8	—	20.3	—
7.9	13,65	15.5	—	—	19.2	—	—	21.3	—	22.0	—
6.0	10.9	11.4	—	—	—	—	—	18.5	—	19.5	—
6.0	8.2	8.9	—	9.7	9.8	—	—	11.9	—	13.0	—
6.0	8.8	—	—	10.4	10.6	—	—	11.5	—	12.0	—
6.0	9.6	11.0	—	—	13.3	—	—	17.7	—	—	—
6.0	—	9.6	—	10.6	10.9	—	—	12.3	—	14.5	—
21	165	270	—	330	440	—	—	560	—	650	610
13	90	170	—	200	260	—	—	430	—	500	500
13	95	150	—	200	210	—	—	340	—	350	360
13	115	190	—	220	270	—	—	450	—	—	—
13	125	190	—	230	290	—	—	430	—	510	520
13	110	180	—	200	240	—	—	340	—	380	390
13	—	115	—	135	150	—	—	—	—	180	190
13	—	240	—	280	330	—	—	420	—	520	580
13	—	195	—	260	320	—	—	420	—	540	560
13	—	190	—	230	330	—	—	420	—	520	580

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inhibit the synthesis of APP. Although an appreciable quantitative difference appeared to exist between the capacity of human and rat erythrocytes for synthesizing APP, no essential difference was observed regarding the influence of the glucose concentrations on APP synthesis. From the experiments described above we must conclude that a definite supply of glucose is necessary to sustain the synthetic capacity of the cells for several hours.

Nevertheless the decrease of the velocity of the synthesis of APP from aneurin during incubation can not solely be explained by the direct influence of the decrease of the glucose concentration on the rate of ATP producing reactions and thus on the rate of APP synthesis. This is proved by the following experiment in which we investigated the influence of the addition of glucose and of aneurin after different times of incubation at 39° C. The set up of this experiment is given in the next diagram; the results are assembled in Table VIII.

	Rat blood	
	16 ml	32 ml
Addition:	1.25 ml isotonic *glucose* solution (5.5%)	2.5 ml isotonic *NaCl* solution (0.9%)
	Incubated at 39° C	
Pipetted from the bloodsamples after 0, 1, 2, 3, 4 and 6 hours' incubation:	2.5 ml	2.5 ml	2.5 ml
Addition:	0.2 ml solution of 12.5 mg aneurin per ml in 0.9% *NaCl* solution	0.2 ml solution of 12.5 mg aneurin per ml in 0.9% *NaCl* solution	0.2 ml solution of 12.5 mg aneurin per ml in *isotonic glucose* solution (5.5%)
	Incubated at 39° C during 3 hours		

In these experiments we see a less pronounced effect of incubation in the absence of aneurin on the capacity for synthesizing APP than in the experiment described in Table VI. (Obviously there are rather large differences between different blood samples with respect to the resistance against incubation in the absence of aneurin.) These experiments further show that even when the blood cells were pre-incubated in a medium rich in glucose the APP-synthesizing capacity gradually decreased. When the blood was pre-incubated without glucose having been added this capacity decreased much faster, but after the first hours of incubation, it could partially be restored, by adding an adequate amount of glucose together with the aneurin, so that it equalled the capacity of blood that had been pre-incubated in a glucose-rich medium.

It is clear however that pre-incubation during 6 hours in the absence of glucose causes some damage to the synthesizing system, which cannot be repaired be adding glucose.

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TABLE VIII

INFLUENCE OF PRE-INCUBATION AT 39° C WITH AND WITHOUT ADDED GLUCOSE ON THE FORMATION OF APP FROM ANEURIN BY RAT BLOOD

Time of pre-incubation (hours)	γ APP per 100 ml after 3 hours' incubation at 39° C with 1 mg aneurin per ml					
	Pre-incubated with 400 mg % glucose		Pre-incubated without glucose			
			Aneurin added only		400 mg % glucose added together with aneurin	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
0	410	415	390	365	410	405
1	400	385	390	370	390	395
½	380	340	320	320	365	360
3	350	340	250	215	350	340
4	340	340	185	165	350	340
6	300	300	125	120	200	130

Summing up we see that during incubation at 39° C alterations occur in the cell's metabolic system, which are only partially prevented by the presence of glucose. The presence of an adequate amount of substrate for the supply of energy is only one of the factors necessary for the maintenance of the metabolic system.

We thus arrive at the general conclusion that the decrease of the rate of APP formation can neither be explained by the decomposition of the added aneurin, nor by the accumulation of inhibitors, the lack of bearer protein or the disappearance of glucose. It can only be explained by a general damage to the metabolic system of the cells during incubation *in vitro* at body temperature which is only accentuated by a lack of substrate. This also explains the decrease of the APP content of the cells in the last period of incubation with aneurin. (Breakdown system of APP less affected than system concerned with APP synthesis).

This work has proved that the production of energy by the dissimilatory processes in the cells is an important factor in the conversion of aneurin into its physiologically active form. However, the possibility of an adequate supply of energy, not only depends upon a sufficient supply of substrate for breakdown, but also on the capacity of the enzymatic system concerned. This depends among others upon the supply of all necessary nutrients, from which we must conclude that the lack of other vitamins or of minerals may invalidate the body's capacity for making use of aneurin. And conversely lack of aneurin might hamper the conversion of other vitamins, into their physiologically active form. So it is conceivable that symptoms pointing to a shortage of one or other of these vitamins might be observed, although they are provided in sufficient amount by the diet. These conclusions might give a clue to the elucidation of the still obscure mechanism of the interrelationships of various avitaminoses.

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SUMMARY

1. The *in vitro* synthesis of aneurinpyrophosphate from added aneurin by the white and red cells of the blood of the rat and of man was studied. Though the synthesis is by no means negligible in human blood it is much higher in rat blood.

2. An average rat leucocyte contains about 160 times as much aneurinpyrophosphate as an average rat erythrocyte. This ratio is increased to about 550 upon incubation of the blood with 1 mg aneurin per ml.

3. The synthesis is inhibited by oxalate, monoiodoacetate, fluoride and cyanide.

4. The synthesis by the red cells is preferentially inhibited by oxalate, the synthesis by the white cells by cyanide. This proves that the energy required for the synthesis of aneurinpyrophosphate from aneurin is mainly provided by glycolysis in the red cells and by respiration in the white cells.

5. Upon incubation of the blood with a small amount of aneurin a higher percentage is converted into aneurinpyrophosphate than with a large amount of aneurin. However, even from 0.1 γ aneurin added to 1 ml of blood only 26% is phosphorylated (from 1 mg added about 0.25% is phosphorylated).

6. During incubation of rat blood with aneurin at 39° C aneurin is not destroyed, nor are substances formed inhibiting aneurin pyrophosphate synthesis.

7. The synthesis of aneurinpyrophosphate by the erythrocytes depends upon the concentration of glucose present. It is decreased when the glucose concentration is below normal or extremely high.

8. The enzym system responsible for the synthesis of aneurinpyrophosphate from aneurin is badly damaged by incubating the blood at 39° C for some hours.

9. Attention is called to the fact that processes producing energy are necessary for the conversion of a certain vitamin into its physiologically active form. As other vitamins form part of various enzymes connected with energy production insight into the interrelationship of various avitaminoses may be gained from investigations on the influence of the lack of one vitamin in the food on the conversion of another vitamin into its physiologically active form.

RÉSUMÉ

1. Nous avons étudié la synthèse *in vitro* du pyrophosphate d'aneurine à partir de l'aneurine sous l'action des érythrocytes et des leucocytes du sang du Rat et de l'Homme. Bien que la synthèse dans le sang humain ne soit pas du tout négligeable, elle est beaucoup plus importante dans le sang du Rat.

2. Un leucocyte de Rat moyen contient environ 160 fois plus de pyrophosphate d'aneurine qu'un érythrocyte de Rat moyen. Cette proportion est augmentée à 550 environ par incubation du sang avec 1 mg d'aneurine par ml.

3. La synthèse est empêchée par l'oxalate, le monoiodoacetate, le fluorure et le cyanure.

4. La synthèse par les globules rouges est surtout empêchée par l'oxalate, la synthèse par les globules blancs par le cyanure. Ceci prouve que l'énergie nécessaire à la synthèse du pyrophosphate d'aneurine à partir de l'aneurine est fournie surtout par la glycolyse dans les globules rouges et par la respiration dans les globules blancs.

5. Par incubation du sang avec une faible quantité d'aneurine un pourcentage plus élevé est transformé en pyrophosphate d'aneurine que par incubation avec une quantité plus grande d'aneurine. Cependant, même si l'on ajoute 0.1 γ d'aneurine à 1 ml de sang 26% seulement en sont phosphorylés (si l'on ajoute 1 mg, 0.25% environ en est phosphorylé).

6. Pendant l'incubation du sang de Rat avec l'aneurine à 39° celle-ci n'est pas détruite et on n'observe pas de formation de substances empêchant la synthèse du pyrophosphate d'aneurine.

7. La synthèse du pyrophosphate d'aneurine par les érythrocytes dépend de la concentration du glucose présent. Elle est diminuée lorsque la concentration du glucose est inférieure au niveau normal ou lorsqu'elle est extrêmement élevée.

8. Le système enzymatique responsable de la synthèse du pyrophosphate d'aneurine à partir de l'aneurine est très affecté par incubation du sang à 39° pendant plusieurs heures.

9. Nous voudrions attirer l'attention sur le fait que des processus producteurs d'énergie sont nécessaires à la transformation de certaines vitamines en leur forme physiologiquement active. Or d'autres vitamines font partie de divers enzymes reliés à la production d'énergie. C'est pourquoi l'étude de l'influence de la carence d'une vitamine donnée sur la transformation d'une autre vitamine en sa forme physiologiquement active pourrait fournir des renseignements sur les relations mutuelles de diverses avitaminoses.

ZUSAMMENFASSUNG

1. Die *in vitro* Synthese von Aneurinpyrophosphat aus Aneurin durch weisse und rote Blutkörperchen der Ratte und des Menschen wurde untersucht. Obwohl die Synthese im menschlichen Blut keineswegs unwesentlich ist, so ist sie doch im Rattenblut viel bedeutender.

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2. Ein Leukozyt enthält im Durchschnitt ungefähr 160 mal soviel Aneurinpyrophosphat wie ein Erythrozyt. Dieses Verhältnis wird durch Inkubation des Blutes mit 1 mg Aneurin pro ml auf 550 erhöht.

3. Die Synthese wird durch Oxalat, Monojodacetat, Fluorid und Cyanid gehemmt.

4. Die Synthese durch rote Blutkörperchen wird vorzugsweise durch Oxalat gehemmt, die durch weisse Blutkörperchen durch Cyanid. Dies beweist, dass die für den Aufbau von Aneurinpyrophosphat aus Aneurin nötige Energie in den roten Blutkörperchen hauptsächlich durch die Glycolyse, in den weissen Blutkörperchen durch die Atmung geliefert wird.

5. Bei der Inkubation des Blutes mit einer kleinen Menge Aneurin wird ein grösserer Prozentsatz in Aneurinpyrophosphat umgesetzt als mit einer grossen Menge Aneurin. Fügt man aber 0.1 γ Aneurin zu 1 ml Blut so werden auch hier nur 26% phosphoryliert (von 1 mg pro 1 ml werden ungefähr 0.25% phosphoryliert).

6. Während der Inkubation von Rattenblut mit Aneurin bei 39° wird das Aneurin nicht zerstört und es werden auch keine Substanzen gebildet, welche die Synthese von Aneurinpyrophosphat hemmen.

7. Die Synthese von Aneurinpyrophosphat durch Erythrozyten hängt von der Glucosekonzentration ab. Diese Synthese nimmt ab, wenn die Glucosekonzentration unter der normalen liegt oder sehr gross ist.

8. Das für die Synthese von Aneurinpyrophosphat aus Aneurin verantwortliche Enzymsystem wird durch Inkubation des Blutes bei 39° während mehrerer Stunden stark beschädigt.

9. Wir möchten darauf aufmerksam machen, dass energieliefernde Prozesse für die Verwandlung gewisser Vitamine in ihre physiologisch aktive Form notwendig sind. Da nun einige andere Vitamine zu gewissen Enzymsystemen gehören, die mit der Produktion von Energie zu tun haben, könnte das Studium des Einflusses des Mangels eines bestimmten Vitamins in der Nahrung auf die Verwandlung eines anderen Vitamins in seine physiologisch aktive Form über das Verhältnis verschiedener Avitaminosen zu einander Aufklärung verschaffen.

REFERENCES

1 T. E. FRIEDEMANN, T. C. KMIECIAK, P. C. KEEGAN, AND B. B. SHEFT, *Gastroenterology*, 11 (1948) 100.
2 R. H. WILLIAMS AND G. W. BISSELL, *Arch. Int. Med.*, 73 (1944) 203.
3 H. G. K. WESTENBRINK AND E. P. STEYN-PARVÉ, *Biochim. Biophys. Acta*, 1 (1947) 87.
4 H. G. K. WESTENBRINK, E. P. STEYN-PARVÉ, A. C. VAN DER LINDEN, AND W. A. VAN DEN BROEK, *Z. Vitaminforsch.*, 13 (1943) 218.
5 G. SMITS AND E. FLORIJN, *Biochim. Biophys. Acta*, 3 (1949) 44.
6 G. A. HARROP AND E. S. G. BARRON, *J. Exp. Med.*, 48 (1928) 207.
7 K. DAMBLE, *Z. ges. exp. Med.*, 86 (1933) 594.
8 R. M. BIRD, *J. Biol. Chem.*, 169 (1947) 493.
9 A. FUJITA, *Biochem. Z.*, 197 (1928) 175.
10 W. F. LOOMIS AND F. LIPMANN, *J. Biol. Chem.*, 179 (1949) 503.

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