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## Energy metabolism during the maturation of reticulocyte in vitro

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# Energy metabolism during the maturation of reticulocyte in vitro\*

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## Abstract

1. For the purpose to clarify the mechanism of the revolutionary changes in energy metabolism during the reticulocyte maturation the metabolisms of glucose and of the pentose moieties of acid soluble nucleotides have been observed on rabbit reticulocytes incubated in vitro under various conditions. 2. The maturation of reticulocyte proceeds by using the energy produced by aerobic glycolysis and is arrested in the glucose deficient medium, but the pentose moieties of purine nucleotide and nucleoside added exogenously serve as the energy source for reticulocyte maturation even in the absence of glucose. 3. The test on the utility efficiency of glucose and inosine as the energy source for reticulocyte maturation revealed that glucose is used more effectively than the pentose moiety of inosine under aerobic condition, which is advantageous for reticulocyte maturation, and vice versa under anaerobic condition, which is comparable to the metabolism of mature red cell. 4. From these results it has been suggested that the maturation of reticulocyte is the process of degradation of RNA and acid soluble nucleotides supported by the aerobic glycolysis, where the degradation products of RNA and acid soluble purine nucleotides provide the purine derivatives as the material for ATP synthesis (36) and the pentose moieties as energy source. 5. A possible mechanism for the superior utility of glucose to nucleoside pentose during reticulocyte maturation and vice versa in mature red cell has been discussed.

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MEDICAL SCHOOL

## ENERGY METABOLISM DURING THE MATURATION OF RETICULOCYTE *IN VITRO*

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The immature anucleate red cell, the mammalian reticulocyte, has all the cytoplasmic components of erythroblast, i. e., mitochondria (1, 2), endoplasmic reticulum or microsomes (2, 3), lysosomes and Golgi apparatus (4). It matures to a red cell by losing gradually all these organellae with an active protein (hemoglobin) synthesis (4, 5). Therefore, the maturation process means a revolutinal change in energy metabolism losing the enzymes of tricarboxylic acid cycle and respiratory system (6—8), with concomitant loss of the enzymes for the *de novo* synthesis of purine nucleotides (9) retaining only low activities of the enzymes of anaerobic glycolysis and pentose cycle (10—12).

The maturation of reticulocyte requires glucose as the energy source (13, 14), while mature red cell forms lactic acid by using purine nucleosides in the absence of glucose (15, 16). According to the observation of NAKAO and his coworkers inosine enhances glycolytic ATP formation of mature red cell in the presence of adenine (17), and TSUBOI has proven that the intracellular level of adenine nucleotides is a limiting factor for the glycolytic process (18), in which the adenine nucleotides are decomposed rapidly to hypoxanthine in the absence of glucose (19).

Similar phenomena were also observed in reticulocytes, i. e., the reticulocyte maturation proceeded with gradual accumulation of hypoxanthine (14) with gradual degradation of its RNA and acid soluble nucleotides. It was reported in the previous paper that the accumulation of hypoxanthine was stimulated by the treatment of respiratory inhibitor and uncoupler of oxidative phosphorylation with the inhibition of RNA degradation (19, 20). These results suggest that in the process of reticulocyte maturation the changes in energy metabolism, which ensues by the gradual loss of aerobic glycolysis, will be closely correlated to the nucleotide metabolism in the cell.

The present experiment was designed to disclose the supposed correlation between acid soluble nucleotide metabolism and glycolysis in the

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revolutional change of energy metabolism during the maturation of reticulocyte and in this paper it was reported that the nucleotides formed by its RNA degradation will be utilized effectively, pentose moieties as the energy source and purine base for ATP synthesis, and the reactions proceed being largely supported by the aerobic glycolysis during the maturation of reticulocyte.

#### MATERIALS AND METHODS

The rabbit red blood cells of phenylhydrazine anemia served as reticulocyte sample as described in the previous paper (21). Throughout the phenylhydrazine injections and the recovery stage red cell count, reticulocyte number, RNA and acid soluble nucleotide contents of a red cell have been observed with the blood (1.0 ml) from ear vein at two days' interval. Reticulocyte counts were taken by the method of SENO *et al.* (22) and RNA and acid soluble nucleotides were extracted by the methods to be described later and the contents were estimated by the absorbancy at 260  $m\mu$ .

When a marked reticulocyte crisis appeared, 3 to 5 days after the last phenylhydrazine injection, 30 ml of blood were drawn from the heart by a syringe containing 3 ml of 3.8 % sodium citrate. The blood was washed twice with ten volumes of 0.85% NaCl (5mM tris-HCl buffer, pH7.4) by repeated centrifugation, removing buffy coat by suction each time. The packed cells sedimented were suspended in the basic medium composed of one volume of normal rabbit serum and 8 volumes of salt solution containing 22.2mM phosphate buffer (pH 7.4), 90 mM NaCl, 700  $\mu$ M KCl, 150  $\mu$ M  $CaCl_2$  and 91  $\mu$ M  $MgCl_2$ . All the procedures for the preparations were carried out at 0° to 4°. The cell suspension so prepared were incubated while shaking gently for varying periods by adding selected concentrations of glucose, inosine 5'-monophosphate (IMP), inosine and ribose 5-phosphate (R5P) at 37°, respectively. Mature red cell suspension was prepared with the blood from healthy animals by the same methods as in the anemic rabbit. The incubation mixture contained 60 to 80  $\times 10^4$  cells per  $mm^3$  in the red cells from the anemic rabbits and 120 to 200  $\times 10^4$  cells from untreated nonanemic rabbits.

For the chemical analysis 0.5 ml of the incubation mixture was taken from each sample at the termination of incubation and frozen quickly in a deep freezer of -20°. All the frozen samples were dissolved by adding cold perchloric acid to the final concentration of 0.5 N, stirred by a glass rod and left standing for one hour at 0° to 4°, and then centrifuged at 15000  $xg$  for 15 minutes. The supernatant was used for the determinations of acid soluble nucleotides, pentose compounds and glycolytic intermediates, and the precipitate for the determination of RNA. The contents of acid soluble nucleotides were estimated by the absorbancy at 260  $m\mu$ , pentose compounds by the orcinol reaction (23), glucose, fructose and their proosphate compounds by the anthrone reaction (24) and lactic acid by the method of BARKER and SUMMERSON (25). RNA content was estimated

by the orcinol reaction (26) after extracting with the modified method of SCHMIDT-THANNHAUSER (20). The amounts of these compounds metabolized by the cells were calculated from the content of each compound at pre- and post-incubation.

## RESULTS

Daily administration of phenylhydrazine induced severe anemia

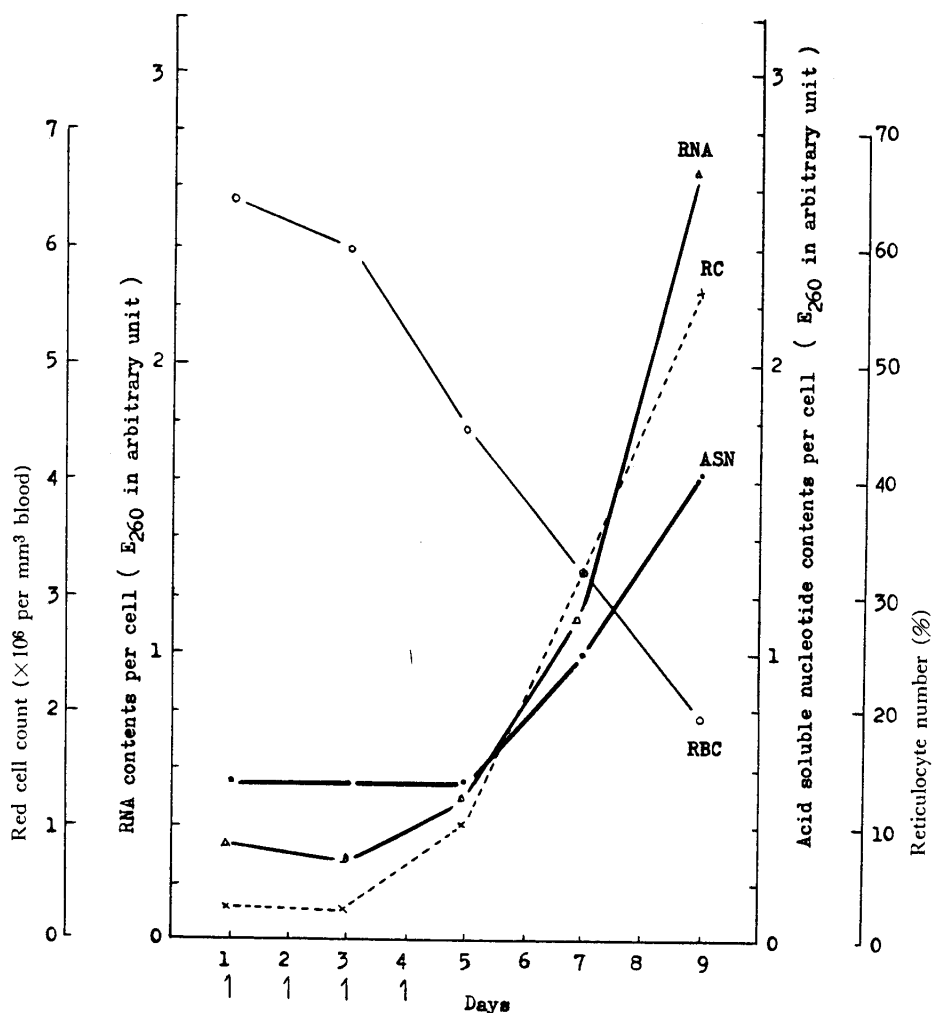


Fig. 1. Daily Changes of Red Cell Count (RBC), Reticulocyte Number (RC), RNA Content (RNA) and Acid Soluble Nucleotide Contents (ASN) in a Red Cell of a Rabbit Receiving 4 Injections of Phenylhydrazine (1.5 ml of 1.5 % phenylhydrazine hydrochloride per day)

The arrows show the phenylhydrazine injections.

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followed by marked reticulocytosis of circulating blood. The contents of acid soluble nucleotides and RNA per cell increased with increase in the reticulocyte number (Fig. 1). The result indicates that reticulocyte contains a large amount of RNA and acid soluble nucleotides but mature red cell does not. That is, the maturation of reticulocyte means the loss of RNA and the diminution of acid soluble nucleotide contents.

In the *in vitro* experiment under aerobic environment the reticulocytes suspended in the medium afore-mentioned decreased in number proportionately with the advance of incubation time. Decreasing curve took the pattern to decline progressively after 8 to 10 hours incubation, indicating that the medium used for the present experiment is suitable for the purpose.

The presence of respiratory inhibitors and an uncoupler or an inhibitor of oxidative phosphorylation stimulated the accumulation of lactic acid more markedly in the media and the successive decrease of acid soluble nucleotides more speedily with the lapse of incubation time (Table 1). The data are comparable to those reported in the previous papers (20, 27), in which the acid soluble nucleotide level was reduced with the accumulation of hypoxanthine and RNA degradation was suppressed by the addition

Table 1 Effects of Respiratory Inhibitors and Uncoupler or Inhibitor of Oxidative Phosphorylation on the Glycolysis and Nucleotide Metabolism of Rabbit Reticulocytes

The cells were incubated in the basic medium containing 3.69 mM glucose and agents shown in the table for 3 hours at 37°. The total volume of incubation mixture, 0.5ml, is 9% in cell volume,  $71 \times 10^4$  cells per mm<sup>3</sup> with 79% reticulocytes at the initiation of incubation. After the incubation the acid soluble compounds were extracted by 0.5 N cold PCA. The contents of glucose, fructose and their phosphate compounds were estimated by the anthrone reaction (24), pentose compounds by the orcinol reaction (23), lactic acid by the method of BARKER and SUMMERSON (25) and nucleotides and bases by the absorbancy at UV wavelength, and the changes of these compounds were presented by  $\Delta$  values which showed the differences of the compounds before and after incubation.

Agent added	(1) $\Delta$ Glucoses ( $\mu$ moles/ ml cells)	(2) $\Delta$ Fructoses ( $\mu$ moles/ ml cells)	(3) $\Delta$ Pentoses ( $\mu$ moles/ ml cells)	(4) $\Delta$ Lactate ( $\mu$ moles/ ml cells)	(5) $\Delta$ Lactate per $\Delta$ Glucose	(6) $\Delta$ E <sub>260</sub> per ml cells	(7) E <sub>250</sub> per E <sub>260</sub>
None	-12.45	+0.58	+0.12	22.2	1.73	+5.78	1.03
57/ml Antimycin A	-21.30	+0.14	-3.06	67.8	3.18	-3.28	1.18
1 mM Amytal	-17.50	+0.29	-1.75	50.2	2.87	-0.45	1.12
100 $\mu$ M 2,4-Dinitrophenol	-28.19	-0.40	-2.83	63.6	2.25	-1.11	1.16
57/ml Oligomycin	-23.28	-0.14	-2.78	69.3	2.98	-2.00	1.18

of similar agents(20). The point to be emphasized is that by three-hour incubation in the presence of respiratory inhibitors and the uncoupler or the inhibitor of oxidative phosphorylation the amount of lactic acid reached a higher level than expected. As shown in Table 1, in the media containing these agents the glucose consumption increased 2 times as high as those of control. The data suggest that some lactic acid should be formed from substrate other than glucose. In this connection, a marked increase in the consumption of pentose compounds may be noted as shown in the third column of the same table. The data show that the metabolism of pentose compounds in reticulocyte is also stimulated by inhibiting ATP formation in mitochondria. A possible source of pentose is the nucleotides and nucleosides, because the 260  $m\mu$ -absorbing materials in the acid soluble fraction decreased markedly with the increase in 250  $m\mu$ -absorbing material by the incubation, indicating the increment of hypoxanthine (20).

Table 2 Utilization of IMP and its Decomposed Products in Relation to RNA Degradation or Maturation of Reticulocyte, Changes in Glycolytic Intermediates and RNA

The cells were suspended in the basic medium added the substrates presented in the table, respectively, and incubated. At the initiation of incubation the incubation mixture was 9 % in cell volume,  $54 \times 10^4$  cells per  $mm^3$  with 49 % reticulocytes. At the selected intervals of incubation time 0.5 ml of incubation mixture were separated respectively and used for the chemical analyses as referred to Table 1. RNA was extracted and estimated from the precipitate, of which acid soluble compounds were removed, by the method as previously described (20).

Substrate added	Incub. time (hr)	$\Delta$ Glucoses ( $\mu$ moles/ml cells)	$\Delta$ Fructoses ( $\mu$ moles/ml cells)	$\Delta$ Pentoses ( $\mu$ moles/ml cells)	+ $\Delta$ Lactate ( $\mu$ moles/ml cells)	- $\Delta$ RNA ( $\mu$ g/ml cells)
5 mM Glucose	1	-13.38	0.00	+2.34	10.82	242
	2	-17.10	0.00	+3.52	21.00	697
	3	-23.45	0.00	+3.57	29.00	928
5 mM IMP	1	-3.01	+0.62	-6.86	9.82	307
	2	-5.08	+1.01	-9.45	16.85	635
	3	-7.28	+1.49	-9.75	25.50	928
5 mM Inosine	1	-1.35	+10.63	-6.65	14.78	336
	2	-1.35	+9.80	-14.76	23.50	670
	3	-3.31	+8.25	-18.65	33.40	1000
5 mM R5P	1	-1.05	+4.85	-9.75	8.53	255
	2	-6.30	+5.67	-10.04	11.09	525
	3	-7.61	+6.29	-10.30	15.15	775
None	1	-3.91	0.00	+0.43	5.28	151
	2	-6.78	+0.06	+1.24	10.34	378
	3	-7.90	+0.06	+1.51	16.62	534

To confirm the availability of pentose moiety of acid soluble nucleotides as the energy source for reticulocyte maturation the effects of exogenous IMP, inosine and R5P on the glycolytic activity and RNA degradation were observed. As shown in Table 2, inosine has a marked stimulating effect for the formation of hexoses and lactic acid and for the degradation of RNA or reticulocyte maturation. IMP also exhibited a similar effects as inosine but the effects were less than that of inosine and were comparable to that of glucose. Besides nucleoside, the added R5P was also consumed with some increase in fructose compounds, but it showed no stimulating effect on the lactic acid formation and RNA degradation. In the media containing no added substrate for energy source the RNA degradation or the reticulocyte maturation was largely arrested but not completely, probably because of the presence of endogenous glucose and nucleotides (Table 2).

The same observation on the mature red cell sample gave similar results observed in reticulocyte suspension, indicating the pentose moiety of inosine and IMP to be an excellent energy source (Table 3). The data are comparable to those obtained by Lowy *et al.* (15).

In the subsequent experiment utility efficiency of glucose and the pentose of inosine was observed from the lactic acid formation in relation to the RNA degradation or cell maturation. At a fixed level of

Table 3 Utilization of IMP and its Decomposed Products by Mature Red Cells, Changes in Glycolytic Intermediates

The experimental conditions were as in Table 2. At the initiation of incubation the incubation mixture was 9% in cell volume,  $131 \times 10^4$  cells per  $\text{mm}^3$  with 1.8% reticulocytes.

Substrate added	Incub. time (hr)	$\Delta$ Glucoses ( $\mu\text{moles/ml cells}$ )	$\Delta$ Fructoses ( $\mu\text{moles/ml cells}$ )	$\Delta$ Pentoses ( $\mu\text{moles/ml cells}$ )	+ $\Delta$ Lactate ( $\mu\text{moles/ml cells}$ )
3.3 mM Glucose	1	-9.13	+2.09	-0.10	9.82
	2	-10.46	+2.09	-0.16	15.90
	3	-11.80	+1.66	-0.22	23.35
5 mM IMP	1	-0.36	+0.59	-1.33	8.67
	2	-1.78	+0.60	-4.23	18.00
	3	-2.90	+0.67	-10.44	26.70
5 mM Inosine	1	-1.90	+9.47	-7.54	13.66
	2	-1.35	+8.79	-17.00	29.00
	3	-1.55	+8.14	-20.70	42.59
5 mM R5P	1	-1.01	+3.04	-3.22	4.67
	2	-2.62	+3.45	-6.90	8.67
	3	-3.76	+3.76	-10.22	10.90
None	1	-0.75	-0.08	-0.28	3.46
	2	-1.87	-0.18	-0.36	8.34
	3	-3.08	-0.10	-0.67	14.10



Table 4 Competitive Utility of Glucose and Inosine by Reticulocyte Suspension, Changes in Glycolytic Intermediates and RNA

The experimental conditions were as in Tables 1 and 2, except substrate concentration which was presented in the table. At the initiation of incubation the incubation mixture was 8.5% in cell volume,  $72 \times 10^4$  cells per  $\text{mm}^3$  with 67.1% reticulocytes. \*: endogenous concentration without any substrate added, \*\*: changes by the incubation with the addition of 5% per ml antimycin A in final concentration.

Prior to incubation		After 3-hour incubation				
Glucose conc. (mM)	Inosine conc. (mM)	$\Delta$ Glucoses ( $\mu\text{moles/ml cells}$ )	$\Delta$ Fructoses ( $\mu\text{moles/ml cells}$ )	$\Delta$ Pentoses ( $\mu\text{moles/ml cells}$ )	+ $\Delta$ Lactate ( $\mu\text{moles/ml cells}$ )	- $\Delta$ RNA ( $\mu\text{g/ml cells}$ )
3.50	0.98	-13.42	0.00	-1.17	33.74	680
3.50	3.48		+4.48	-8.26	37.84	644
3.50	5.98	-12.60	+8.96	-11.78	35.95	644
3.50	10.98	-12.50	+10.96	-7.64	35.74	621
1.00	5.98	-4.59	+9.32	-11.19	38.64	660
2.00	5.98	-14.10	+8.47	-10.71	38.64	612
6.00	5.98	-13.60	+8.13	-5.54	37.74	612
11.00	5.98	-13.42	+7.66	-2.20	37.94	644
* 1.00	* 0.98	-5.57	-0.54	-4.83	20.24	560
6.00	5.98	** -30.36	** +6.49	** -16.72	** 93.14	** 460

Table 5 Competitive Utility of Glucose and Inosine by Mature Red Cell Suspension, Changes in the Glycolytic Intermediates

The experimental conditions were as in Table 4, except substrate concentration which is presented in the table. At the initiation of incubation the incubation mixture was 13.5% in cell volume,  $200 \times 10^4$  cells per  $\text{mm}^3$  with 0.6% reticulocytes.

Prior to incubation		After 3-hour incubation			
Glucose conc. (mM)	Inosine conc. (mM)	$\Delta$ Glucoses ( $\mu\text{moles/ml cells}$ )	$\Delta$ Fructoses ( $\mu\text{moles/ml cells}$ )	$\Delta$ Pentoses ( $\mu\text{moles/ml cells}$ )	+ $\Delta$ Lactate ( $\mu\text{moles/ml cells}$ )
5.79	0.36	-17.05	+0.19	+1.48	37.22
5.79	1.36	-15.20	+0.37	-3.73	48.20
5.79	5.36	-11.53	+4.95	-12.80	48.30
3.29	0.36	-13.54	-0.37	+0.79	32.42
3.29	1.36		-0.45	-5.35	40.13
3.29	5.36	-9.49	+3.75	-13.61	46.24
0.79	0.36	-5.34	-0.55	-0.24	15.60
0.79	1.36	-4.28	-0.36	-6.41	26.58
0.79	5.36	-4.21	+3.61	-16.15	44.50

glucose concentration, 3.5 mM, with varying concentration of inosine, 0.98 to 10.98 mM, the consumption of the pentose of inosine increased to some extents with the increase of the inosine concentration in the media with a slight change of glucose consumption, and at a fixed level of inosine, 5.98 mM, with varying concentrations of glucose, 1.0 to 11.0 mM, the pentose consumption was increased with the decreasing concentration of glucose. The consumption of glucose remained at a certain level inspite the changed glucose concentration in the media (Table 4). In both series of experiments, the rate of lactic acid formation was retained at a certain level showing no significant change, and the RNA degradation or reticulocyte maturation proceeded nearly at the same rate, indicating that either one, glucose or purine nucleoside pentose, is utilized effectively as the energy source for the cell maturation, compensating each other. The level of fructose compounds was considerably increased concomitant with raising of inosine concentration in the presence of glucose and slightly decreased with the rise in glucose concentration. In the reticulocytes the consumption of glucose and inosine was stimulated with the increase in lactic acid formation with the inhibition of respiration by antimycin A; about twice in glucose consumption and 3 times in pentose consumption, with 2.5 times in lactic acid formation comparing to the values found in the reticulocytes incubated without antimycin A under oxygenated environment.

Similar observation was made by using mature red cells which had no mitochondria and ensued themselves an anaerobic glycolysis (Table 5). The observations revealed that the mature red cells also consume both glucose and pentose of inosine forming lactic acid as in reticulocytes. The difference lies in that in reticulocytes the glucose consumption is kept nearly constant even though the inosine concentration in the media is increased, while in mature red cells the glucose consumption is decreased when the inosine concentration is increased and *vice versa*.

From the data of the consumption rate of substrate per cell (Table 6), it is noted that reticulocyte shows about 8.5 times in glucose consumption and 4.5 times lactic acid formation as that of mature red cell when glucose is used as substrate. In the case where inosine was used as substrate reticulocyte exhibits 6.5 times the consumption of pentose of inosine and 4 times lactic acid formation as that of mature red cell. Namely, in the process of reticulocyte maturation the revolutionary change of energy metabolism from aerobic to anaerobic glycolysis proceeds with the diminution of metabolic activity and the diminution rate is higher in the pathway consuming glucose than in the pathway consuming pentose.

Table 6 Comparison of Activities of Glucose and Inosine Consumption and of the Formation of Lactic Acid between a Reticulocyte and a Mature Red Cell

Experimental conditions were as in Table 2 except of cell sample. The values presented in the table were calculated by dividing each compound with cell number in the sampling mixture. The values of each activity per reticulocyte were obtained by the following equation;  $a^* = (b \cdot n' \cdot c) / n$ , here,  $a^*$ : the activities of a reticulocyte to consume glucose and inosine and to form lactic acid respectively,  $b$ : the average activity per cell in reticulocyte suspension,  $c$ : the average activity per mature red cell,  $n$ : the average number per cent of reticulocyte in the reticulocyte suspension (55%) and  $n'$ : the average number per cent of mature red cell in reticulocyte suspension (45%). \*\*: Each of the average activity per cell was calculated by neglecting the numbers of reticulocyte in the mature red cell suspension. \*\*\*: Actual value should be 4.5 instead of 1.87, because  $5.7 \times 10^{-9}$   $\mu$ moles lactic acid were formed, probably consuming pentoses from the RNA degradation products. \*\*\*\*: calculated as  $b = 4.5$  instead of 1.87.

Cell count	Per cent of reticulo. at the start of incubation	Substrate added at the initiation of incubation					
		5 mM Glucose			5 mM Inosine		
		$-\Delta$ Glucose per cell (10 <sup>-9</sup> $\mu$ moles)	$+\Delta$ Lactate per cell (10 <sup>-9</sup> $\mu$ moles)	$+\Delta$ Lactate per $-\Delta$ Glucose	$-\Delta$ Pentose per cell (10 <sup>-9</sup> $\mu$ moles)	$+\Delta$ Lactate per cell (10 <sup>-9</sup> $\mu$ moles)	$+\Delta$ Lattate per $-\Delta$ Pentose
Reticulocyte suspension							
71	68.5	2.42	3.94	1.63	1.36	4.16	3.06
67	64.3	4.96			2.39	7.90	2.40
77	57.5	3.56	4.74	1.33	1.22	4.86	2.40
52	50.0	2.65			1.42	6.17	4.35
43	50.0	5.50	7.95	1.44	2.26		
52	42.7	3.77	4.89	1.30	2.56	5.37	2.02
average	55.0	3.82	5.03	1.43	1.87***	5.70	3.16
Mature red cell suspension							
200	0.6	0.80	1.74	2.18	1.09	2.08	1.81
118	1.6	0.69	1.60	2.32	1.42	2.95	2.08
116	0.1	0.73	1.67	2.29	0.80	1.70	2.10
average**		0.74	1.67	2.26	1.10	2.24	2.00
average activity per reticulocyte (*a)		6.3	7.7		7.2****	8.6	

#### DISCUSSION

The maturation of reticulocyte or RNA degradation with protein synthesis is retarded under anaerobic environment (28) or in the presence of the agents to arrest the respiration or oxidative phosphorylation (20, 29). On the other hand, the maturation process means the gradual decomposition of mitochondria (4), which results inevitably in the loss of the enzymes carrying on respiration and oxidative phosphorylation (6—8). This will mean that reticulocyte requires a quantity of ATP formed mainly

by mitochondrial oxidative phosphorylation and with the advance of maturation accompanied by the diminution of protein synthesis, incidentally excess of mitochondria will be decomposed.

It has been reported that the maturation of reticulocyte *in vitro* is arrested in the glucose deficient medium (13, 14), but the present observation indicated that the reticulocyte maturation proceeds without any arrest in the glucos deficient medium when the medium contains inosine or IMP (Tables 2 and 4). The result indicates that the reticulocyte will use purine nucleotide and nucleoside pentose as energy source as well as glucose.

Question may arise which is more effective as energy source, glucose or purine nucleoside pentose. The experiment revealed that the consumption of the pentose moiety of inosine decreased with the increasing concentration of glucose in the media and that the glucose consumption was slightly decreased by the increase of added inosine (Table 5). The results indicate that glucose is superior to pentose of inosine as the energy source for reticulocyte maturation. The mechanism of the superiority of glucose to pentose as an energy source in the reticulocyte may be explained by the fact that the phosphorylation of inosine to ribose 1-phosphate (30, 31) competes with that of fructose-6-phosphate to fructose 1,6-diphosphate (32, 33), for inorganic phosphate under high activity of hexokinase (EC, 2. 7. 1. 1) in reticulocytes (12). The data suggest that the activity of phosphofructokinase (EC, 2. 7. 1. 11) is also higher in reticulocyte than in mature red cell (Tables 4 and 5). On the other hand, the pentose moieties of purine nucleotide and nucleoside are consumed markedly with the accumulation of lactic acid and hypoxanthine, and yet the rate of the consumption of nucleoside pentose to that of glucose increased by the additions of respiratory inhibitors and the uncoupler or inhibitor of oxidative phosphorylation (Tables 1 and 4). The results indicate that the mechanism which controls the superior activity of glucose consumption to pentose moieties in reticulocyte is released by inhibiting mitochondrial ATP synthesis and the mechanism approaches closely to that of mature red cell (Tables 4 and 5).

In the mature red cell, which has no mitochondria and only ensues anaerobic glycolysis, the glucose consumption decreases with the increase in the added inosine in the media under a fixed concentration of glucose (Table 5). This may be due to the fact that the activities of hexokinase (12) and phosphofructokinase (32) are lowered in the rather acidic environment containing accumulated lactic acid and glycolysis is inhibited at the steps of these enzyme reactions, while the enzymes responsible for the

transformation of purine nucleotide to hexose and triose compounds, i. e. . AMP deaminase (AMP aminohydrolase, EC. 3. 5. 4. 6) (34) and purine nucleoside phosphorylase (purine nucleoside; orthophosphate ribosyltransferase, EC. 2. 4. 1. 1) (30), are rather active in acidic environment. This supposition is supported by the fact that the diminution rate in the pathway consuming glucose is higher than in the pathway consuming pentose during the revolutionary changes of energy metabolisms in the process of reticulocyte maturation (Table 6) and also supported by the fact that the diminution in the rate of lactic acid formation by adding glucose as the energy source *in vitro* in the acidic environment (pH 7.1) is higher than by adding inosine in the reticulocyte suspension (Table 7).

Table 7 Changes of Glycolytic Activity of Reticulocyte Suspension by Adding Glucose and Inosine in Relation to the pH of the Incubation Mixture

The experimental conditions were as in Table 1 except substrate and 50 mM phosphate buffer solution. At the initiation of incubation the incubation mixture was 11.0% in cell volume,  $118 \times 10^4$  cells per  $\text{mm}^3$  with 52.6% reticulocytes.

Prior to incubation		After 3-hour incubation				
Substrate added	pH of medium	pH of medium	$\Delta$ Glucoses ( $\mu\text{moles}/\text{ml cells}$ )	$\Delta$ Fructoses ( $\mu\text{moles}/\text{ml cells}$ )	$\Delta$ Pentoses ( $\mu\text{moles}/\text{ml cells}$ )	+ $\Delta$ Lactate ( $\mu\text{moles}/\text{ml cells}$ )
5.59mM Glucose	6.80	7.10	-15.82	-0.74	+5.42	25.39
	7.20	7.35	-24.74	-0.66	+5.22	34.30
	7.50	7.45	-25.65	-0.13	+4.34	42.10
	7.80	7.60	-32.20	+0.06	+2.81	47.65
	8.20	7.70	-30.10	+0.14	+2.90	54.90
5.00mM Inosine	6.80	7.10	-8.01	+5.09	-10.33	42.50
	7.20	7.30	-9.75	+3.97	-10.52	52.40
	7.50	7.35	-12.10	+4.48	-16.35	53.51
	7.80	7.60	-13.24	+3.77	-16.70	55.60
	8.20	7.65	-9.01	+4.14	-13.41	56.30

The data suggest that such a process may occur with the advance of reticulocyte maturation where its mitochondria are largely lost. Thus, reticulocyte maturation proceeds with the RNA degradation which provides the pentose moieties of purine nucleotides as energy source and the purine base for ATP synthesis (35, 36). These revolutionary changes in energy metabolism will proceed by the aid of mitochondria under aerobic environment consuming glucose as well as its own purine nucleotides. When the reticulocyte matures finally losing mitochondria the process of respiratory ATP synthesis is completely lost and the pentose moieties of purine nucleotides are used preferentially as energy source than glucose (19).

#### SUMMARY

1. For the purpose to clarify the mechanism of the revolutionary changes in energy metabolism during the reticulocyte maturation the metabolisms of glucose and of the pentose moieties of acid soluble nucleotides have been observed on rabbit reticulocytes incubated *in vitro* under various conditions.

2. The maturation of reticulocyte proceeds by using the energy produced by aerobic glycolysis and is arrested in the glucose deficient medium, but the pentose moieties of purine nucleotide and nucleoside added exogenously serve as the energy source for reticulocyte maturation even in the absence of glucose.

3. The test on the utility efficiency of glucose and inosine as the energy source for reticulocyte maturation revealed that glucose is used more effectively than the pentose moiety of inosine under aerobic condition, which is advantageous for reticulocyte maturation, and *vice versa* under anaerobic condition, which is comparable to the metabolism of mature red cell.

4. From these results it has been suggested that the maturation of reticulocyte is the process of degradation of RNA and acid soluble nucleotides supported by the aerobic glycolysis, where the degradation products of RNA and acid soluble purine nucleotides provide the purine derivatives as the material for ATP synthesis (36) and the pentose moieties as energy source.

5. A possible mechanism for the superior utility of glucose to nucleoside pentose during reticulocyte maturation and *vice versa* in mature red cell has been discussed.

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