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Abstract

The effects of high fatty acids such as oleic, richinoleic, linoleic, linolenic, palmitic and stearic acids, on the respiration, glycolysis, organic phosphate synthesis of Ehrlich ascites tumor cells, were studied. The unsaturated fatty acids added to the media enhanced the respiration of the tumor cells at the concentration lower than 0.2 mM, after a short incubation period and inhibited the respiration in a high concentration 0.4 mM. The saturated fatty acids did not show such effect. All the fatty acids, both of saturated and unsaturated, effected the increase in lactate formation in tumor cells, especially markedly at higher concentration being accompanied by the WQ increase and RQ around 1. The respiration lowered by the fatty acids was ameliorated by the addition of glucose. The lactate formation from glucose was greatly enhanced by the addition of fatty acids but hardly from pyruvate. The unsaturated high fatty acids proved to have a strong uncoupling action for oxidative phosphorylation. This effect could be recognized slightly in the saturated fatty acids. The addition of high fatty acid resulted in the striking decrease in ATP and ADP with the increase in AMP. With these results the discussion was conducted concerning the specificity of tumor cell related to the glucose and fatty acid metabolism.

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THE EFFECTS OF HIGH FATTY ACID ON THE GLUCOSE METABOLISM OF EHRLICH ASCITES TUMOR CELLS*

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One of the specificities of cancer cell may be found in its respiratory metabolism on the endogenous substrate[1-11]. This endogenous respiration of cancer cell is something of longstanding comparing with that of normal cell, e. g. the respiration of Ehrlich ascites tumor cell frequently lasts more than 6 to 10 hours with slow decrease in activity[12,13] as observed *in vitro*, whereas the respiration of normal cell, mouse liver cell, lasts only 2 to 3 hours at most under the same condition. However, the respiration of tumor cell is hardly accelerated by glucose or the members of citric acid cycle but it decreases by adding glucose or fructose differing from the case of normal cell whose respiration is generally enhanced by adding glucose or the members of citric acid cycle. This phenomenon is well known as Crabtree effect.

For a long time this effect has been studied by many biochemists and is currently understood as the result of extraordinarily accelerated cytoplasmic glycolysis in cancer cell which will result out of the compartmentation in ADP and P*i* between cytoplasm and mitochondria[9,14,16], and then the decreased respiration. However, it might be noticed that in cancer cells the endogenous respiration is largely dependent on fatty acid oxidation, more than 30 per cent as WEINHOUS and associates[10] stated. Their studies on lipid metabolism using ^{14}C-palmitate, also show that more than one half of energetic metabolism of cancer cell is dependent upon lipids[7,17]. This specific metabolism of cancer cell might be correlated with Crabtree effect. The free fatty acids added from the outside of cells may act as an uncoupling agent on the oxidative phosphorylation accelerating the respiration at their lower concentrations, differently from the endogenous fatty acid (SCHOLEFIELD[9,18] and LEHNINGER[19], etc.).

Our studies revealed the enhancing effect of saturated and unsaturated fatty acids for the glycolysis and suppressing effect for oxidative phosphorylation of tumor cells. These facts suggest a close connection between glucose and fatty acid metabolism in tumor cell. In this paper the effects of fatty acids on glycolytic metabolism of Ehrlich ascites tumor cell are presented.

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MATERIALS AND METHODS

Ehrlich ascites tumor cells, growing in C_3H mice in an ascitic form, were used. The ascites tumor cells are harvested 8—9 days after transplantation and are washed 3 times with physiological saline solution by the repeated centrifugation to remove the blood elements contaminated. Centrifugation can be carried out most effectively by raising to 2, 000 r. p. m. within several seconds and switching off just after reaching 2, 000 r. p. m. Thus the majority of tumor cells precipitate and blood cells remain in supernatant. The precipitate is resuspended in Krebs-Ringer solution (KR soln.) of about 8 volumes of the precipitate.

The fatty acids to be added to the tumor cell suspension are those of oleic, ricinoleic, linoleic and linolenic as unsaturated acids, and stearic and palmitic as saturated ones. These fatty acids are mixed with 1/20 volume of Tween 80 respectively and emulsified by adding some physiological saline solution so as to prepare a colloidal solution of fatty acid, 3.6 *m M* in concentration. Waring Blender homogenizer is used for the preparation of the colloidal solution. With this colloidal solution fatty acid solutions in varying concentration, 0.90 to 3.6 *mM*, are prepared by using 0.9 per cent saline solution, and 0.3 ml. each is added to cell suspension of 2.5 ml.

The respiration, glycolysis and phosphorylation of cancer cells are measured by the routine method with Warburg's apparatus, by using the following media: For aerobic glycolysis; 0.3 ml. of 0.1 *M* Na-phosphate buffer solution (pH 7.4) and 1.9 ml. of the cell suspension in main chamber, 0.2 ml. of 20 % KOH in center well, and 0.3 ml. of 0.18 *M* glucose (0.9 % NaCl for control) and 0.3 ml. of 0.9 to 3.6 *m M* fatty acid in side arm. For anaerobic glycolysis; 2.1 ml. of the cell suspension in KRP solution (pH 7.4) in main chamber, 0.2 ml. of H_2O in center well, 0.3 ml. of 0.18*M* glucose (0.9 % NaCl for control) and 0.3 ml. of 0.9 to 3.6 *m M* fatty acid (0.9 % NaCl for control) in side arm *a* and 0.1 ml. of 5 *N* H_2SO_4 in side arm *b*.

For the estimation of the ^{32}P incorporation into organic phosphate compounds; 1.0 ml. of the cell suspension in Ca free KR solution in main chamber, 0.2 ml. of 20 per cent KOH in center well, 0.3 ml. of 0.18 *M* glucose (0.9 % NaCl for control) and 0.3 ml. of 0.9 to 3.6*m M* of fatty acid (0.9 % NaCl for control) in side arm *a* and 0.7 ml. of KRP solution and 0.5 ml. of 0.02*M* Na_2HPO_4 labelled with ^{32}P (20μc) in side arm *b*.

The incubation is carried out at 38°C for 60 minutes with air for aerobic glycolysis and with nitrogen gas containing 5 per cent CO_2 for anaerobic glycolysis, and at 38°C for 30 minutes with air for ^{32}P incorporation into organic phosphate. After the incubation aerobic glycolysis is terminated by adding 0.3 ml. of 5 *N* H_2SO_4 for manometric estimation and by rapid cooling to 0°C

for chemical analysis, the quantitative estimation of lactic acid. The method of BARKER-SUMMERSON[20] is applied for lactic acid

In the case of anaerobic glycolysis the chemical analyses have been carried out on lactate by the same method as in aerobic glycolysis, and ^{32}P incorporations into Δ10P fraction (organic labile phosphate compounds) are observed on the cell after the proposed incubation. The reaction is stopped by rapid cooling to 0°C after the incubation and the cells are washed with cold KR solution several times by repeated centrifugation.

The quantitative estimation of phosphate has been made by TAKAHASHI's method[21], ^{32}P counting on the inorganic phosphate and Δ10P by using G-M counter (Kobe Kogyo 131 type).

Dehydrogenase activities of the cell are measured by TUNBERG's method by which the levels of the enzyme activities are determined by the time required for decoloration of methylene blue. The incubation mixture used in this method is as follow; 2.0 ml. of 0.1 *M* Na-phosphate buffer (pH 7.4), 1.0 ml. of 0.18*M* glucose, 0.6 ml. of 3.6 *m M* fatty acid (the same volume of 0.9 % NaCl for control) and 1.0 ml. of 0.01 per cent methylene blue in main chamber, and 1.0 ml. of the cell suspension in Ca free KR solution in side arm. The tube is set after filling in the chambers with the mixture and the cell suspension respectively, and then the air is drawn by water-flow aspirator followed by the introduction of N_2 gas. After repeated drawing and N_2 gas introduction 3 times, the cell suspension in side arm is added to the main chamber and the decoloration time of methylene blue is measured at 25°C.

Estimation of acid soluble phosphate compounds has been carried out on the fractions separated with column chromatography. Incubation mixture is composed of 4.0 ml. of cell suspension in KR solution suspended 1 g. of the packed cells, 0.5 ml. of 3.6 *m M* oleic acid and 0.5 ml. of 0.02 *M* Na-phosphate buffer (pH 7.4) in KR solution and incubated for 30 minutes at 25°C. For the test series fatty acid is added to the mixture in 0.2 *m M* as final concentration and for the control 0.9 per cent NaCl instead of fatty acid. After incubation the cells are precipitated by centrifugation, the supernatant is decanted, and the cells in precipitate are frozen by dry ice acetone bath. Then the acid soluble phosphate fractions are extracted from the packed cells with 5 per cent perchloric acid, adsorbed on the column of Dowex 1 × 4 formate form (200-400 meshes) and eluted with sodium formate and ammonium formate solutions applying the technique of TERADA[22].

In each experiment the observation has been conducted three times and the mean values are recorded.

RESULTS

O_2 consumption and lactate formation of Ehrlich ascites tumor cells in the media containing fatty acids : The increased respiration of tumor cells at short incubation period has been observed on those incubated with high fatty acids, oleic, linoleic, linolenic, parmitic and stearic acids in the final concentration of 0.05 to 0.2 *mM*. In the higher concentration than 0.2 *m M*, however, the respiration has been inhibited by the fatty acids, though there is some enhanced O_2 uptake at initial stage. The lactic acid formation of the tumor cells has always been enhanced by the presence of these high fatty acids, showing the increased lactate formation with the increased fatty acid concentration, 0.4 *m M* in the highest. In concentration of 0.4 *m M* of fatty acid the amount of lactate reached 2 to 2.5 times the values of the control. In Table 1 the relation between the lactate formation

Table 1. The effect of varying fatty acids concentrations on the respiration and lactic acid formation of Ehrlich ascites tumor cells

System	Ratio of lactate formation				Ratio of Q_{O_2}			
	Oleic	Ricinoleic	Linoleic	Linolenic	Oleic	Ricinoleic	Linoleic	Linolenic
−Glu. − FA					100	100	100	100
−Glu. +0.1mM					112	68.1	—	100
−Glu. +0.2mM					98	36.6	91.0	100
−Glu. +0.4mM					32.2	12.8	44.5	34.2
+Glu. − FA	100	100	100	100	69.5	60.0	59.0	58.5
+Glu. +0.1mM	164	204	99.4	103	76.5	51.6	57.5	54.0
+Glu. +0.2mM	179	174	107	103	73.6	31.0	61.5	57.0
+Glu. +0.4mM	213	186	148	153	47.2	18.8	51.1	48.8

of tumor cells and O_2 consumption in the medium having each fatty acid may be seen. In the higher concentation O_2 consumption is inhibited markedly whereas the lactate formation is extremely enhanced by fatty acids, but in the lower concentration the fatty acids do not inhibit the O_2 consumption, though the lactate formation is enhanced. Lactate formation, Q_{CO_2}, RQ, WQ, Q_{O_2}, Q_L^{air} and $Q_L^{N_2}$ of the tumor cells at 0.4 *mM* concentation of each fatty acid have been presented in Table 2, and the effects of oleic acid in the varying concentation on O_2 uptake of the cell are presented in Fig. 1 showing the specific type of curve of "fall-off" in the higher concentration of the fatty acid. The effect is very marked in the absence of glucose (Table 2). Of course, without adding glucose the lactate production is small and not detectable by the method applied. The Crabtree effect is observable in these tumor cells, too, as the decreased respiration by adding glucose to the incubation mixture (Fig. 1). This effect

Table 2. Effects of fatty acids 0.4 *mM* on the respiration and the glycolysis of Ehrlich ascites tumor cells

System	Ratio of lactate formation	Q_{O_2}	Q_{CO_2}	RQ	WQ	Q_L^{air}	$Q_L^{N_2}$
−Glu. −FA		4.9	4.36	0.89			
−Glu. +oleic		2.14	2.58	1.21			
−Glu. +ricinoleic		1.22	0.78	0.64			
−Glu. +linolic		1.63	1.76	1.08			
−Glu. +linolenic		2.23	1.76	0.79			
−Glu. +palmitic		4.38	3.82	0.89			
−Glu. +stearic		4.63	4.19	0.91			
+Glu. −FA	100 (0.437)*	2.71	2.71	0.95	3.84	10.05	16.98
+Glu. +oleic	173	2.16	2.37	1.10	8.80	17.40	18.70
+Glu. +ricinoleic	114	1.29	1.89	1.40	9.77	11.58	17.68
+Glu. +linoleic	203	1.50	1.59	1.06	14.90	20.40	17.50
+Glu. +linolenic	188	2.50	2.55	1.02	8.28	18.88	15.52
+Glu. +palmitic	165	2.97	3.00	1.01	6.27	16.55	21.80
+Glu. +stearic	129	3.04	2.89	0.95	4.62	12.99	17.36

* : (μ moles/mg of cells/60 min.)

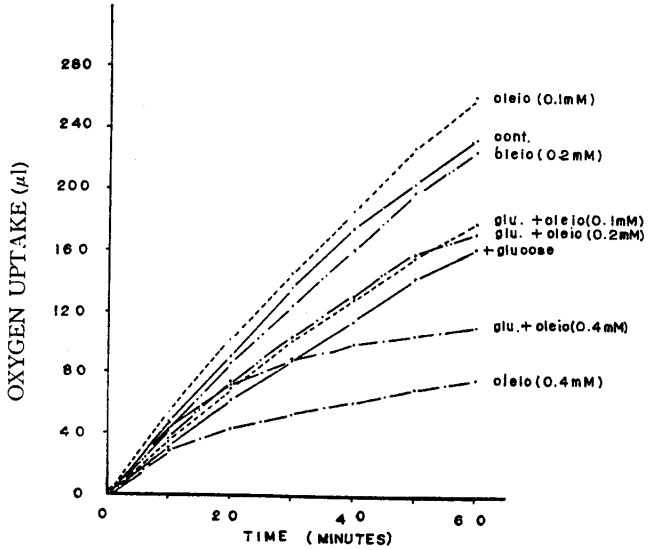

Fig. 1. Effect of oleic acid on the oxygen uptake of Ehrlich ascites tumor cell

is reduced in the presence of fatty acids, in the range of the concentration of 0.1 to 0.2 *mM*. All the four unsaturated fatty acids tested show similar properties.

But the saturated fatty acids, parmitic and stearic, prove to have no such

an activity even at 0.4 *mM* concentration. In spite of the restoring effect the lactate formation seen in the glucose containing media is rather enhanced by the unsaturated fatty acid, though the effect of saturated fatty acids is much less. WQ values, the ratio of lactate formation to O_2 consumption, are increased and RQ approaches 1 or goes over.

In these experiments the changes in pH of the media should be taken into consideration, as the addition of the fatty acids will act as to lower the pH which might give some influence on the lactate formation and respiration of the cells. The experiments by using the media whose pH is adjusted to pH 7.2 to 5.6, however, show only a slight decrease in respiration and a marked decrease in the lactate formation with the decreased pH values. The data show that the increased lactate formation and respiration by the addition of fatty acid cannot be induced by the reduced pH values of the media (Fig. 2).

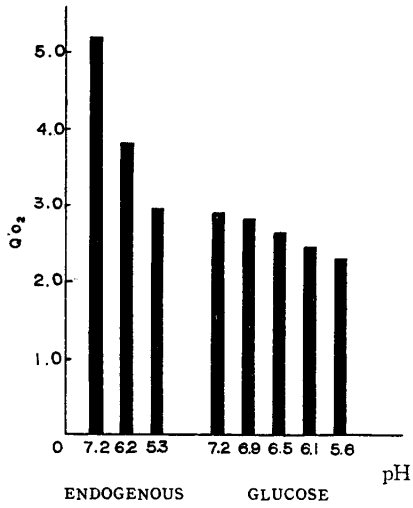

Fig. 2. Effect of pH on the O_2 uptake of Ehrlich ascites tumor cells

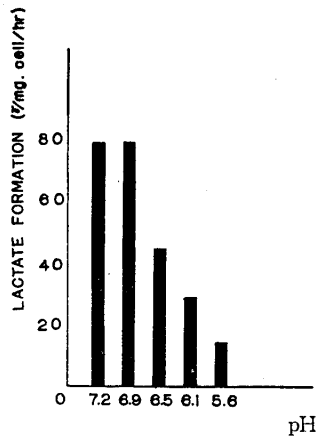

Fig. 2'. Effect of pH on the anaerobic glycolysis of Ehrlich ascites tumor cells

The effect of pyruvate : By the β-oxidation mechanism the fatty acid is finally oxidized to acetyl-CoA which is also produced by aerobic glycolysis. Acetyl-CoA is led to citrate being condensed with oxalacetic acid by the condensing enzyme. Citric acid is oxidized to carbon dioxide and water through the pathway of the tricarboxylic acid cycle. Keeping in mind this process is reasonably presumed that concerning CoA, the competition may occur between the two processes of glycolysis and fatty acid oxidation. If this is true, excess pyruvate and fatty acid in the medium would enhance the lactate formation from pyruvate. Therefore, 0.02 *M* pyruvate was added to Ehrlich ascites tumor cell

suspension and the oxygen consumption and lactate formation were investigated with or without adding oleic acid, 0.1 to 0.4 *mM* in concentration (Table 3).

Table 3. The effect of oleic acid on the respiration and lactic acid formation of Ehrlich ascites tumor cells with pyruvate as a substrate

System		Lactate formation (μ *moles*/mg)	Q_{O_2}
−Pyruvate	−FA	0.059	4.34
+Pyruvate	−FA	0.271	4.34
+Pyruvate	+0.1 mM	0.281	5.60
+Pyruvate	+0.2 mM	0.285	5.31
+Pyruvate	+0.4 mM	0.295	3.10

The results showed that the addition of pyruvate enhanced the lactate formation but further addition of fatty acid brought not so remarkable increase in lactate formation as in the addition of glucose, indicating that there is not so remarkable competition concerning CoA between the two ways for glycolysis and fatty acid oxidation, as far as Ehrlich ascites tumor cells are concerned. Therefore, actually no recognizable competition of fatty acid and pyruvate on CoA.

Dehydrogenase activity of the tumor cells : As just mentioned the respiration of Ehrlich ascites tumor cells is slightly inhibited by adding 0.4 *mM* of unsaturated fatty acids but in the early stage of the incubation the O_2 consumption is invariably enhanced. This initial enhancement of O_2 consumption may be associated with the increased activity of dehydrogenase. The actual measurement proved the enhanced dehydrogenase activity of the cells by adding all sorts of fatty acids tested, especially by oleic acid as shown in Fig. 3.

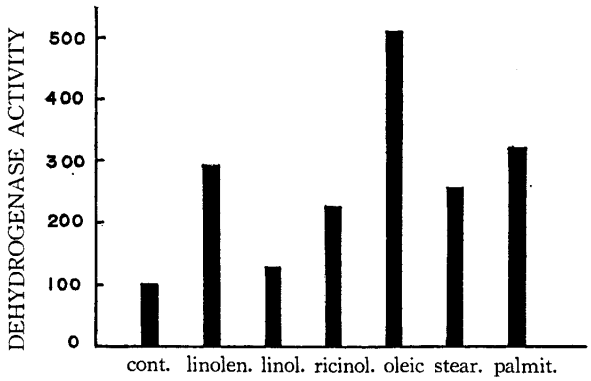

Fig. 3. Effect of fatty acid on the dehydrogenase activity

Increased concentration of fatty acids acts as to prolong the decoloration time. Data obviously suggest that oxygen consumption is closely correlated with the activity of dehydrogenase.

- ^{32}P incorporation into the organic phosphate compounds : As is already

known, decanoate[18] and oleate[19] act as an uncoupling agent for oxidative phosphorylation like DNP, but no informations are available concerning other fatty acids. And the authors observed the effect of several unsaturated and saturated fatty acids on the ^{32}P incorporation into acid soluble organic phospate compounds, especially ATP on Ehrlich ascites tumor cells. As illustrated in Table 4, the incorporation of ^{32}P into the △10P fraction is markedly inhibited by high fatty acids, particularly by unsaturated fatty acids, but in the case of stearic acid the inhibition of the incorporation is slight in the form of free acid and marked in the sodium salt. This inhibited ^{32}P incoporation is ameliorated considerably by the addition of glucose to the incubation mixture, suggesting the enhanced

Table 4. Effect of fatty acid on the respiration and the incorporation of ^{32}P into △10P fraction of Ehrlich ascites tumor cells

System	Ratio of O_2 uptake	Ratio of R. A. of Pi	Ratio of R. A. of △10P	Ratio of SA of △10P
− Glu. − FA	100	100	100	100
− Glu. + linoleic	44	111	6	55
− Glu. + oleic	27	51	5	76
− Glu. + stearic	94	99	83	96
− Glu. + Na-stearate	42	40	8	40
+ Glu. − FA	59	62	319	301
+ Glu. + linoleic	36	73	175	159
+ Glu. + oleic	34	29	100	148
+ Glu. + stearic	66	60	302	300
+ Glu. + Na-stearate	55	23	237	198

ATP regeneration by glycolysis. Table shows the inhibited O_2 uptake by adding fatty acid. This has never been seen in the former observations. But in this experiment SCHOLEFIELD'S system[18] for incubation was applied. The data differing from the former experiment are probably due to the difference in the composition of the medium.

Acid soluble fraction of the tumor cells: The distributional changes among the members of acid soluble fraction of the Ehrlich ascites tumor cells may be expected by the treatment with various high fatty acids, as it is known that some surface active agents act as the uncoupler for oxidative phosphorylation and activate ATPase[23] of the cell as well. The column chromatogram of acid soluble fractions from Ehrlich ascites tumor cells incubated with oleic acid, 0.2 *mM*, exhibited the low peaks of ATP and ADP and high one of AMP (Fig. 4). This can clearly be seen when compared with the result obtained on the control experiment where the cells were incubated with NaCl solution instead of the fatty acid (Fig. 5).

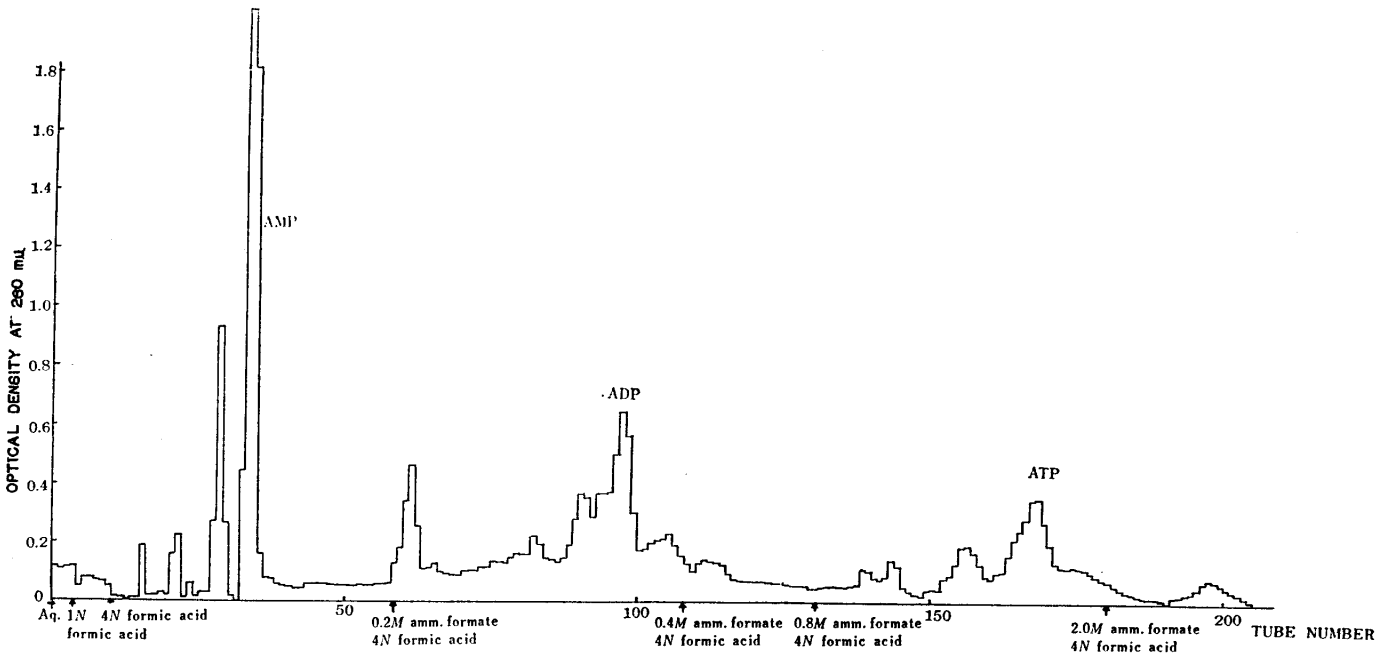

Fig. 4. A column chromatography of acid-soluble fraction in Ehrlich ascites tumor cells after incubation for 30 minutes with KRP solution containing 0.4 *mM* oleic acid

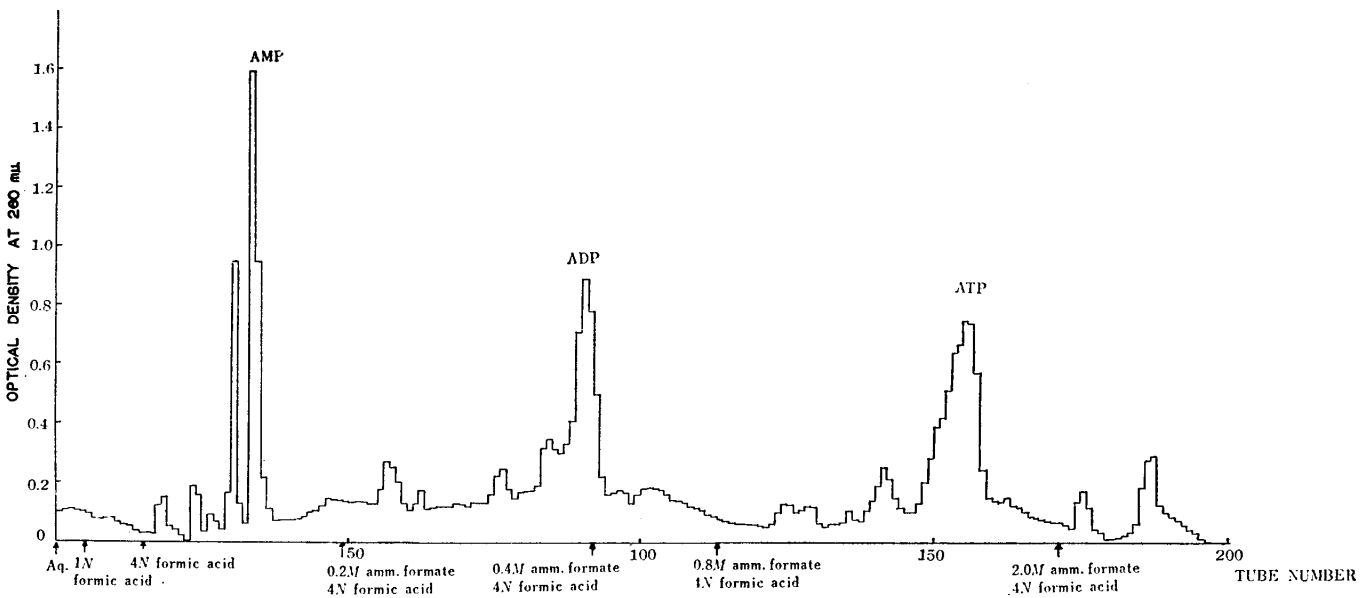

Fig. 5. A column chromatography of acid-soluble fraction in Ehrlich ascites tumor cells after incubation for 30 minutes with KRP solution.

DISCUSSION

From the studies on the endogenous respiration, lipid and glucose metabolisms of cancer cells and the body cells of tumor bearing animals, a marked deviation of their metabolism from those of normal cells have been revealed by several authors[24]. The works also show that the deviations are represented by those in the metabolism of carbohydrates and lipids, i. e. tumor cells catabolize lipids, probably as their energy source, depriving them from the host cells. This use of lipids as the energy source may act as to reduce the RQ of tumor cells, but unexpectedly the fatty acids given from the outside of the cells *in vitro* have effected to increase the RQ of tumor cells. This curious effect of the exogenous fatty acids might be specific to the malignant tumor cells, because in normal liver cells its RQ can never be increased by the exogenous fatty acids but always decreased. The phenomenon may be due to the uselessness of the exogenous fatty acid as the energy source for tumor cells but the work of WEINHOUS demonstrated the exogenous palmitate-^{14}C can be metabolized by cancer cells as well[7]. The metabolism of palmitate, however, can be inhibited by glucose, fructose, lactate or acetate, any one of which is used by tumor cells in presence of palmitate[7]. Data also show that the inhibition of fatty acid metabolism by glucose and others is only possible in long chain fatty acids. The inhibition will mean some competition in the utilization of CoA between the process of fatty acid oxidation and glycolysis. Another explanation, however, is that the reduction process enhanced by the elevated anabolism of tumor cells by adding fatty acid may be responsible for the increase in RQ. In the authors' experiments the lactate formation from the glycolysis is remarkably accelerated by adding high fatty acids. This phenomenon seems to support the view that CoA is used for fatty acid oxidation and the metabolism of glucose to citrate is intercepted by the relative deficiency in CoA which will result in the increase in lactate formation. If this supposition is true, lactate formation from exogenous pyruvate should also be increased by adding fatty acid. However, the experiment showed only a slight increase in lactate formation by fatty acid. The results indicate that competition in CoA between fatty acid oxidation and glycolysis will not be responsible for the enhanced lactate formation, at least in the major portion of it, seen in tumor cells after adding glucose and fatty acids. The results also show that the increased lactate formation observable at addition of fatty acid will be due to some unknown mechanism on the way of glycolysis to pyruvate. One of the possibilities is that uncoupling mechanism for oxidative phosphorylation by high fatty acid will be responsible for the phenomenon. That is, the uncoupling for oxidative phosphorylation will result in an increase in ADP. ADP may be turned to AMP and

ATP by adenylate-kinase. The ATP will act as to accelerate the process of glucose-6-phosphate (G-6-P) formation from glucose by hexokinase. Further on the way of D-glyceroaldehyde-3-phosphate to 1, 3-diphospho-D-glycerate DPN is reduced to DPNH, which is coupled to lactate formation from pyruvate. Then the reasonable explanation of the accelerated lactate formation is in glycolysis but not by adding pyruvate (Fig. 6). For this supposed mechanism of increased lactate

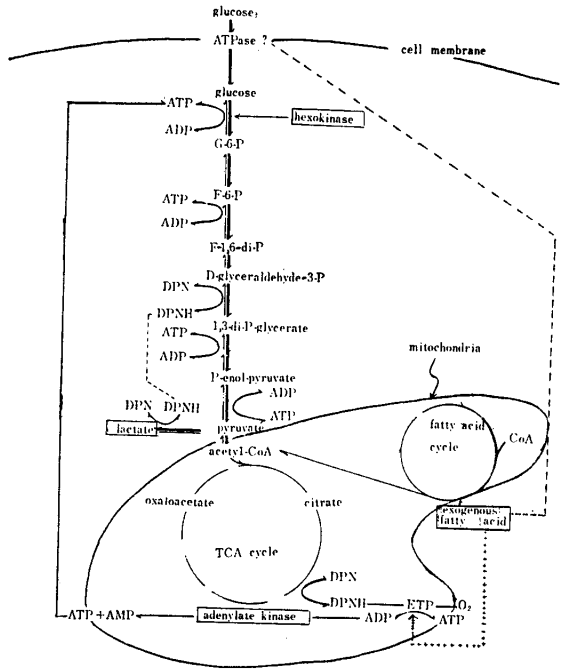

Fig. 6. Diagrammatic representation of metabolic control of glycolysis and fatty acid oxidation by oxidative phosphorylation

formation in tumor cells by fatty acids several evidences have been accumulated. As has been clearly indicated, the fatty acids proved to be the uncoupling agent for oxidative phosphorylation in Ehrlich ascites tumor cells, where 0.2—0.4 *mM* free high fatty acids acted as to inhibit the ATP synthesis strikingly. As pointed by PRESSMAN[23] the fatty acids accelerate the activity of latent ATPase of mitochondria. Such an action of fatty acid will result in the accumulation of ADP in the cell. The column-chromatographic analysis of the acid soluble fraction from tumor cells incubated with fatty acids proved the decrease in ATP and increase in AMP. This suggests the decomposition of ATP to ADP and further the latter to ATP and AMP by adenylate-kinase. ATP is further used for the G-6-P formation. Repeating this process, AMP alone will increase finally. Con-

sequently, the fatty acids added to the cell from outside will induce the ATP deficiency and act as to impair the cell function when glucose reserve is small.

But if some glucose is supplied, the cell will escape from the damage as can be supposed from the data appearing in Table 4. This will occur in normal cells as well as in tumor cells, though the former will show the increased glycogen synthesis and the latter the lactate formation, as the tumor cells are low in G-6-Pase and draw the main energy from anaerobic glycolysis. According to the McKEE's data[9] 8 per cent of glucose is oxidized by TCA cycle in Ehrlich ascites tumor cell. Consequently, in tumor cells the decrease in ATP from TCA cycle by fatty acids will be compensated by that from the increased glycolysis. SCHOLEFIELD have also found the fatty acids act as uncoupling agent for oxidative phosphoryation in tumor cell and this effect is reduced by adding glucose[18]. From these data they suggested some correlation between glucose and fatty acid metabolisms which would be governed by oxidative phosphorylation[8,25]. Independently from this work, LEHNINGER extracted so-called U-factor[26] from the aged or warm incubated mitochondria, from rat liver, which has the characteristics as the uncoupling activity for oxidative phosphorylation. This U-factor has been confirmed to be a kind of free fatty acids[26].

All these data including the present works seem to show that free fatty acids may be the regulator for the energy metabolism in cancer cell, though there is several points left to be clarified to establish this concept. Prior to being uptaken by the cell, the free fatty acids may combine with protein forming lipoprotein. This lipoprotein has been proven to be easily metabolized[25,27], with a markedly reduced uncoupling activity, i. e. the uncoupling reaction of free fatty acids, both of endogenous and exogenous, is inhibited by adding serum albumin[19,26] with which the fatty acids form lipoprotein[28]. This suggests that the uncoupling by fatty acids may be induced by the changes of the mitochondrial structure whose protein may combine with the lipid[29,30] and then degeneration of mitochondria, though it requires morphologic observation to settle this point.

SUMMARY

The effects of high fatty acids such as oleic, richinoleic, linoleic, linolenic, palmitic and stearic acids, on the respiration, glycolysis, organic phosphate synthesis of Ehrlich ascites tumor cells, were studied. The unsaturated fatty acids added to the media enhanced the respiration of the tumor cells at the concentration lower than 0.2 *m M*, after a short incubation period and inhibited the respiration in a high concentration 0.4 *mM*. The saturated fatty acids did not show such effect. All the fatty acids, both of saturated and unsaturated, effected the increase in lactate formation in tumor cells, especially markedly at

higher concentration being accompanied by the WQ increase and RQ around 1. The respiration lowered by the fatty acids was ameliorated by the addition of glucose. The lactate formation from glucose was greatly enhanced by the addition of fatty acids but hardly from pyruvate. The unsaturated high fatty acids proved to have a strong uncoupling action for oxidative phosphorylation. This effect could be recognized slightly in the saturated fatty acids. The addition of high fatty acid resulted in the striking decrease in ATP and ADP with the increase in AMP. With these results the discussion was conducted concerning the specificity of tumor cell related to the glucose and fatty acid metabolism.

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