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TURNOVER RATE OF PLASMA FREE FATTY ACIDS IN COLD-ACCLIMATED RATS

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Summary Effects of norepinephrine infusion on rectal temperature, plasma FFA concentration and its turnover rate were studied in intact and functionally eviscerated rats.

Norepinephrine infusion caused a greater increase in the rectal temperature, and less elevation in plasma FFA concentration in cold-acclimated rats in comparison with the changes in warm-adapted control rats. Norepinephrine infusion also brought about a significant increase in the turnover rate of plasma FFA in both groups of rats. The regression coefficient of plasma FFA turnover rate *versus* FFA concentration was greater in cold-acclimated group than in the controls. It was assumed that peripheral capacities of FFA uptake were greater in cold-acclimated rats than in the controls.

The thermogenic effect of norepinephrine in eviscerated rats did not differ from that in non-eviscerated rats. The infusion caused an increase in the rectal temperature in eviscerated cold-acclimated rats, but not in warm-adapted ones. Norepinephrine infusion produced a similar marked elevation in plasma FFA concentration in both eviscerated cold-acclimated and warm-adapted rats, while increase in FFA turnover rate was much greater in the former group than in the latter. The regression coefficient of plasma FFA turnover rate on its concentration was greater in eviscerated cold-acclimated rats than in the controls.

There is little doubt that plasma levels of free fatty acids (FFA) control the rate of their uptake by the mass-action effect (ARMSTRONG *et al.*, 1961a, b; SPITZER and GOLD, 1964). Plasma FFA turnover rate was shown to vary with the plasma concentration in a straight-line fashion and it was suggested that the regression coefficient of this straight-line relation is affected by changes in the status of energy requirement (ISSEKUTZ *et al.*, 1964; MINAIRE *et al.*, 1973; PAUL and HOLMES, 1973). As to the plasma FFA turnover rate in cold-acclimated

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animals, so far only one study has been reported by McBURNEY and RADOMSKI (1969) who found that fractional turnover rates of FFA, of very low-density lipoprotein and of low-density lipoprotein, were higher in cold-acclimated rats than in the corresponding controls.

We previously observed that in cold-acclimated rats norepinephrine infusion caused a significant elevation in rectal temperature, while in warm-adapted control rats the temperature decreased. In the former group, however, the extent of increase in the plasma FFA level during norepinephrine infusion was much less when compared with the changes in the latter group (MORIYA *et al.*, 1974). The greater elevation in rectal temperature and the lesser increase in plasma FFA in cold-acclimated rats might be explained by the increased turnover rate of FFA in these animals, since the thermogenic effect of norepinephrine, which is known to mediate nonshivering thermogenesis in cold-acclimated rats (HSIEH and CARLSON, 1957; HSIEH *et al.*, 1957; DEPOCAS, 1960; MORIYA *et al.*, 1973a, b), seems to be related to its lipolytic action (HANNON and LARSON, 1962; STEINBERG *et al.*, 1964; HAVEL *et al.*, 1964). Furthermore, the regression coefficient of the relationship between plasma FFA levels and turnover rates in cold-acclimated rats may differ from that in the controls. In order to ascertain these inferences, the turnover rate of plasma FFA was measured simultaneously with FFA concentration and rectal temperature in cold-acclimated rats under the same conditions as in our previous experiments (MORIYA *et al.*, 1974).

In addition to these measurements in intact rats, studies were also performed on functionally eviscerated rats. It is generally accepted that some part of circulating FFA is utilized directly by peripheral tissues (GEYER *et al.*, 1951; FRIEDBERG and ESTES, 1962), but 30 to 50% is removed from the blood by the liver (STEINBERG, 1964; BASSO and HAVEL, 1970) where it is rapidly esterified and a part of this fraction reappear in the blood mainly in association with the very low-density lipoprotein. Peripheral tissues, especially skeletal muscles, are considered to be more contributable to nonshivering thermogenesis than central organs in cold-acclimated rats (DEPOCAS, 1958; JANSKY and HART, 1963), although contradictory consequences have been also presented (FELTS and MASORO, 1959; HANNON *et al.*, 1963). Studies on eviscerated rats might be worth-while for studying the role of muscles in the thermogenic effect of norepinephrine in cold-acclimated rats.

METHODS

Male Wistar rats, weighing from 250 to 400 g, were used in the present experiments. The animals were acclimated to cold by exposing them to a temperature of 5°C for 2 to 4 weeks, while control rats were kept at 22°C. They were provided with water and a chow (Oriental MF) ad libitum.

^{14}C-U-palmitate (New England Nuclear Corp., specific activity: 500 Ci/mole) was prepared for infusion by dissolving its sodium salt in distilled water and

diluting with an equal volume of 10% solution of bovine serum albumin (Armour Pharm. Co., Fraction 5).

After 18 hr fasting, rats were anesthetized with urethane (150 mg/100 g body weight, s.c.). A continuous infusion of ^{14}C-palmitate-albumin complex solution was given *via* the right femoral vein using a constant infusion pump which delivered 14 μl of solution (^{14}C: 5 mμCi) per min through an indwelling polyethylene catheter. Blood samples were taken from the jugular vein or carotid artery into heparinized syringes at intervals as indicated below. These samples were provided for measurements of plasma FFA concentration and its specific activity (SA). A plateau in FFA–SA was obtained within the first 10 min and thereafter it remained almost constant for at least 50 min. The first blood sample was drawn at 20 min after the ^{14}C-palmitate infusion. Immediately after the sampling norepinephrine (Sankyo Pharm. Co.) infusion was started, concurrently with ^{14}C-palmitate infusion *via* another femoral vein at a constant rate of 2 μg per min. In this case, a plateau in FFA–SA was also obtained within 10 min and remained constant for at least 40 min during continuing norepinephrine infusion. The second blood sample was drawn 30 min after the norepinephrine infusion. Under the steady state condition, the infusion rate of ^{14}C-palmitate divided by SA of FFA represents turnover rate of plasma FFA (ARMSTRONG *et al.*, 1961a).

Plasma FFA concentration was determined by the method of DUNCOMBE (1963). Radioactivity in the FFA fraction was counted according to the method used by ARMSTRONG *et al.* (1961a), employing toluene containing 0.4% PPO and 0.01% POPOP as scintillater.

Rectal temperature was measured by the use of a thermister thermometer at a site of 5 cm from the anus. Local temperature of interscapular brown adipose tissue was also measured by a thermister.

Functionally eviscerated rats were prepared by ligating rectal colon, superior mesenteric and coeliac arteries and portal vein. Before the operation glucose was subcutaneously injected in a dose of 100 mg/kg body weight in order to maintain blood glucose at nearly constant level. In these rats the second blood sample was drawn 20 min after the norepinephrine infusion.

RESULTS

1. Changes in rectal temperature by norepinephrine infusion

Effect of intravenous infusion of norepinephrine at a rate of 2 μg/min for 30 min on the rectal temperature was first observed in cold- and warm-acclimated rats under urethane anesthesia. Effect of saline infusion was also surveyed as control.

Before the infusion rectal temperatures were on the same level in these two groups. In the case of saline infusion, a gradual lowering of the rectal temperature was observed in both cold-acclimated and control rats (Table 1). On the other

Table 1. Changes in rectal temperature during norepinephrine infusion (2 μg/min) in rats under urethane anesthesia.

	before	Δ15 min	Δ30 min
Warm-adapted control rats	(°C)		
saline infusion (8)	36.87±0.21	−1.11±0.15	−2.00±0.33
norepinephrine (15)	36.18±0.25	−0.33±0.11	−0.51±0.19
Cold-acclimated rats	36.88±0.21		
saline infusion (6)		−0.69±0.11	−1.24±0.16
norepinephrine (15)	36.49±0.41	+0.52±0.13	+1.11±0.26

Mean±SEM. Numbers in parentheses represent number of rats.

hand, norepinephrine infusion resulted in a significant elevation of the rectal temperature in the both groups. However, cold-acclimated group responded to norepinephrine with a much greater increase in the temperature than controls did. The average increase was 2.4°C for the cold-acclimated rats, while it was 1.5°C for the controls. This, it is apparent that the calorigenic action of norepinephrine is far greater in the cold-acclimated rats than in the controls.

2. Changes in plasma FFA concentration and its turnover rate by norepinephrine infusion

Effects of norepinephrine infusion on the plasma FFA concentration and its turnover rate were examined in the cold-acclimated and control rats. Before the infusion were not different of the two groups. Norepinephrine infusion caused significant increase in the values of each group, respectively. After 30 min plasma FFA concentration elevated to 992 μEq/liter±38 (SEM) in the cold-acclimated rats and to 1185±46 in the control rats. Values of FFA turnover rates were 4.21 μEq/min/100 g body weight ±0.31 and 3.39±0.19, respectively (Table 2).

Table 2. Changes in plasma FFA level and turnover rate produced by norepinephrine infusion (2 μg/min for 30 min) in rats under urethane anesthesia.

	Warm-adapted	Cold-acclimated	*P*
No. of rats	14	16	
Body weight, g	310	350	
FFA level, μEq/liter			
Before	580±22	626±30	NS
After	1185±46	992±38	<0.01
Difference	605±42*	366±29*	<0.001
FFA turnover rate, μEq/min/100 g body weight			
Before	1.99±0.13	2.29±0.21	NS
After	3.39±0.19	4.21±0.31	<0.05
Difference	1.39±0.14*	1.92±0.22*	NS

Mean ± SEM. NS: not significant * $P<0.001$

It should be noted that in the cold-acclimated rats less elevation of plasma FFA concentration was observed in spite of the greater increase in its turnover rate during the infusion.

When a relationship between plasma FFA concentration and its turnover rate was examined, significant correlations were obtained in the both groups (Fig. 1). The relations were given as $Y=0.0047\ X-0.582$ ($r=0.753$, $P<0.01$) for the

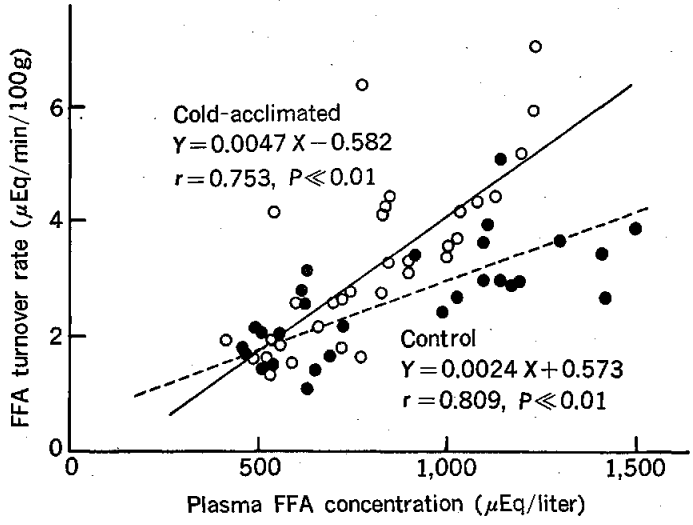


Fig. 1. Relationship between plasma FFA concentration and its turnover rate in cold-acclimated (open circles) and control (closed circles) rats.

cold-acclimated group and $Y=0.0024\ X+0.573$ ($r=0.809$, $P<0.01$) for the controls, and the regression coefficient for the cold-acclimated group was significantly greater than that for the controls ($P<0.05$).

3. Elevation of rectal temperature in relation to plasma FFA concentration and its turnover rate

In order to investigate the possibility that the elevation of rectal temperature induced by norepinephrine infusion is related to the increase in plasma FFA concentration or its turnover rate, the values of temperature were plotted against the levels of plasma FFA or turnover rate. As shown in Figs. 2 and 3, positive correlations were obtained between rectal temperature and logarithm plasma FFA concentration or logarithm FFA turnover rate. For the plasma FFA concentration the relations were given as $Y=7.122 \log X+16.79$ ($r=0.644$, $P<0.01$) in the cold-acclimated group and $Y=3.973 \log X+25.19$ ($r=0.378$, $P\fallingdotseq0.05$) in the controls, and for the FFA turnover rate $Y=5.018 \log X+34.90$ ($r=0.674$, $P<0.01$) and $Y=6.577 \log X+34.01$ ($r=0.648$, $P<0.01$), respectively.

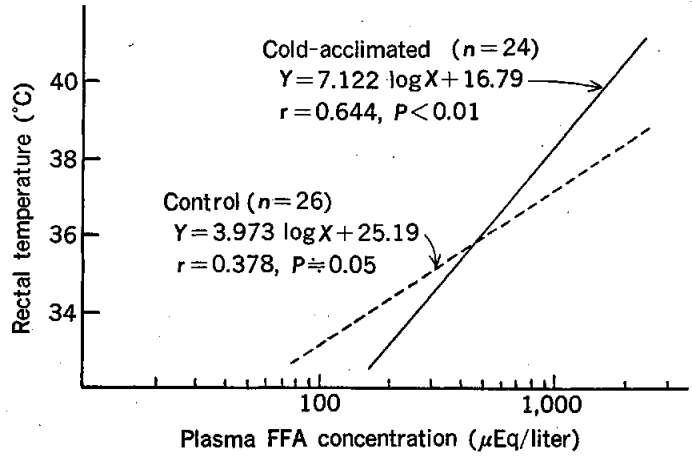


Fig. 2. Relationship between plasma FFA concentration and rectal temperature in cold-acclimated and control rats.

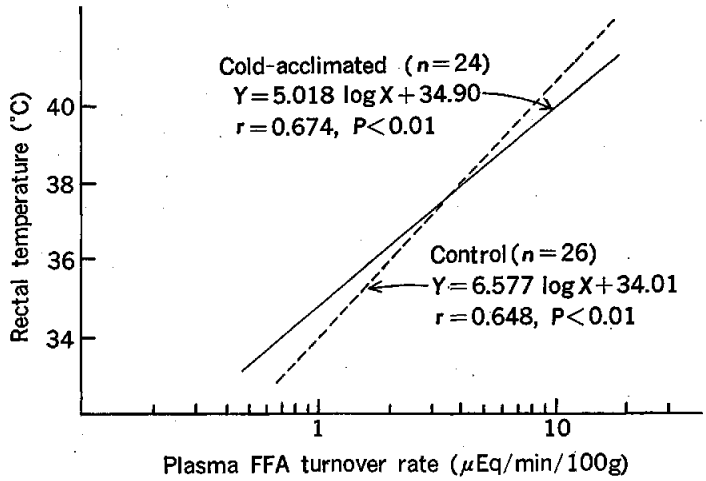


Fig. 3. Relationship between plasma FFA turnover rate and rectal temperature in cold-acclimated and control rats.

4. Changes in plasma FFA concentration and its turnover rate by norepinephrine infusion in functionally eviscerated rats

In functionally eviscerated rats, effect of norepinephrine infusion on rectal as well as interscapular brown adipose tissue (BAT) temperature was examined. Although evisceration caused a lowering of temperature, before the infusion the rectal temperature of eviscerated cold-acclimated rats was on the same level as that of eviscerated controls. Norepinephrine was found to cause a significant increase in the rectal temperature in the former group, but not in the latter one (Table 3).

Furthermore, the effect of norepinephrine on BAT temperature in eviscerated rats did not differ from that in intact rats (Table 4). This indicated that the thermogenic response to norepinephrine in BAT is not injured by evisceration.

Table 3. Changes in rectal temperature during norepinephrine infusion (2 μg/min) in eviscerated rats under urethane anesthesia.

		Body weight	Before	Δ 20 min
		(g)	(°C)	
Warm-adapted rats	(5)	305	32.26±0.50	−1.29±0.19
Cold-acclimated rats	(5)	308	33.06±0.21	+0.26±0.20
P				<0.01

Mean ± SEM. Numbers in parentheses represent number of rats.

Table 4. Changes in local temperature of interscapular brown adipose tissue produced by norepinephrine infusion (2 μg/min) in normal and eviscerated rats under urethane anesthesia.

	Body weight	Before	Change
	(g)	(°C)	(30 min)
Normal			
Warm-adapted			
saline infusion (8)	335	35.8±0.33	−1.6±0.19
norepinephrine (9)	320	34.8±0.22	0±0.19
Cold-acclimated			
saline infusion (4)	295	35.8±0.27	−1.0±0.17
norepinephrine (6)	300	34.9±0.46	+2.8±0.45
			(20 min)
Eviscerated			
Warm-adapted			
saline infusion (4)	295	32.7±0.44	−1.7±0.19
norepinephrine (5)	305	32.6±0.60	−0.2±0.14
Cold-acclimated			
saline infusion (4)	370	33.2±0.26	−2.0±0.19
norepinephrine (5)	310	33.5±0.07	+1.5±0.29

Mean ± SEM

After the evisceration plasma FFA turnover rate was markedly reduced in the both groups, whereas its concentration was relatively unchanged. These values were not different each other in the both groups. Twenty minutes after norepinephrine infusion plasma FFA concentration increased to 1826 μEq/liter±96 (SEM) in eviscerated cold-acclimated rats and to 1723±110 in eviscerated warm-adapted rats, while FFA turnover rates were 3.24 μEq/min/100 g body weight±0.49 in the former group and 1.61±0.16 in the latter group ($P<0.01$). The results are summarized in Table 5.

When relations between plasma FFA concentration and its turnover rate obtained in these two groups were compared, it was found that the regression coefficient from eviscerated cold-acclimated group was greater than that from eviscerated controls ($P<0.05$), as seen in Fig. 4. The relations were give as $Y=$

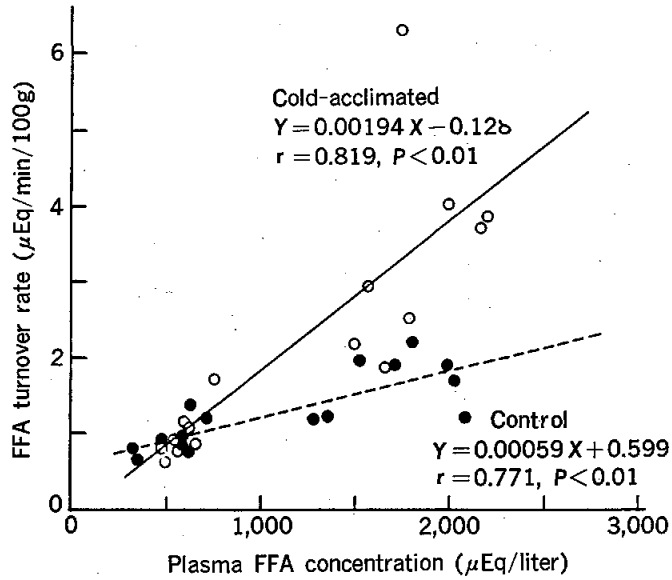


Fig. 4. Relationship between plasma FFA concentration and its turnover rate in eviscerated cold-acclimated (open circles) and control (closed circles) rats.

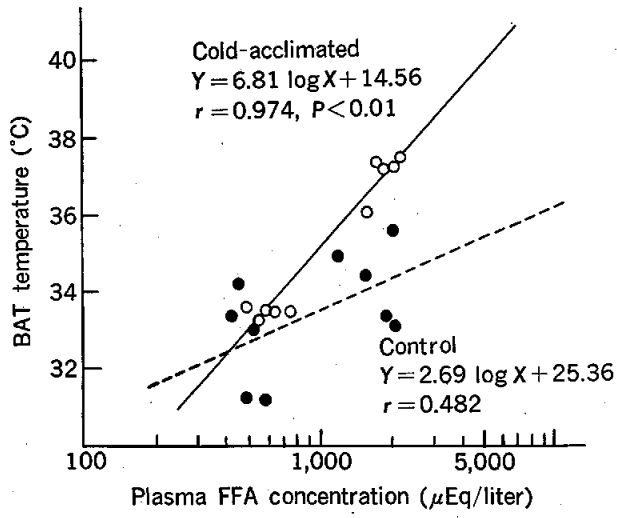


Fig. 5. Relationship between plasma FFA concentration and brown adipose tissue temperature in eviscerated cold-acclimated (open circles) and control (closed circles) rats.

Table 5. Changes in plasma FFA level and turnover rate produced by norepinephrine infusion (2 μg/min for 20 min) in eviscerated rats under urethane anesthesia.

	Warm-adapted	Cold-acclimated	*P*
No. of rats	8	8	
Body weight, g	305	320	
FFA level, μEq/liter			
Before	518±46	584±32	NS
After	1723±110	1826±96	NS
Difference	1205±99*	1225±81*	NS
FFA turnover rate, μEq/min/100 g body weight			
Before	0.90±0.09	1.01±0.12	NS
After	1.61±0.16	3.42±0.49	<0.01
Difference	0.71±0.11*	2.41±0.48*	<0.01

Mean ± SEM. NS: not significant. * $P<0.001$

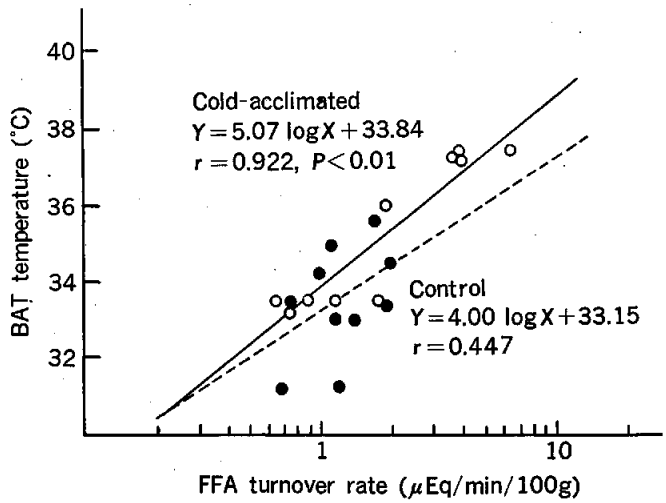


Fig. 6. Relationship between plasma FFA turnover rate and brown adipose tissue temperature in eviscerated cold-acclimated (open circles) and control (closed circles) rats.

0.00194 log X−0.128 in the former group and Y=0.00059 log X+0.599 in the latter group. Furthermore, positive correlations were observed between BAT temperature and plasma FFA concentration (Fig. 5) and plasma FFA turnover rate (Fig. 6) in eviscerated cold-acclimated rats, but no significant correlations were seen in controls.

DISCUSSION

According to HSIEH *et al.* (1966), in cold-acclimated rats anesthetized, curariz-

ed and under artificial respiration the regression coefficient of logarithm increase in oxygen consumption induced by norepinephrine infusion on logarithm dose of norepinephrine was significantly greater than that from warm-adapted rats. FFA concentration and increase in oxygen consumption correlated well with dose of norepinephrine and period of cold exposure. In control rats a positive correlation was found between plasma FFA concentration and oxygen consumption, whereas in cold-acclimated rats a negative correlation was observed. From the results they suggested that the increase in magnitude of the response to norepinephrine in cold-acclimated rats could not be accounted for by assuming an increase in sensitivity to norepinephrine alone, and therefore that an increase in the capacity of the systems utilizing oxygen and substrate may also be considered.

In the present study, after norepinephrine infusion rectal temperature and plasma FFA turnover rate were higher in cold-acclimated rats than in control ones, whereas plasma FFA concentration was lower in the former group than in the latter group. Furthermore, the regression coefficient of plasma FFA turnover rate on its concentration was greater in cold-acclimated rats than in controls. Between FFA turnover rates and concentrations there observed significant positive correlations in these two groups and the intersection of these two correlation curves was at 502 μEq/liter for FFA concentration and at 1.78 μEq/min/100 g body weight for FFA turnover rate around which resting levels were ordinarily found in the both groups (Fig. 1). From the above results it might be assumed that peripheral capacities of FFA uptake which might be activated directly or indirectly by norepinephrine were greater in cold-acclimated rats than in controls. In spite of the fact that lipolytic response to norepinephrine is enhanced in white adipose tissue of cold-acclimated rats *in vitro* (THERRIAULT *et al.*, 1969; MITCHELL and LONGWELL, 1964), less increase in plasma FFA concentration was observed in cold-acclimated rats after norepinephrine. This finding supports the above inference that the FFA uptake by peripheral tissues increased in cold-acclimated rats. In this regard, LAFRANCE *et al.* (1972) observed in chronically norepinephrine-treated rats lower levels of plasma FFA and higher oxygen consumption response following norepinephrine administration, as compared with the responses in controls.

Furthermore, in the both groups positive significant correlations were demonstrated between rectal temperature and plasma FFA turnover rate and no difference was observed in the two groups (Fig. 3). Therefore, in these groups an equal percentage of plasma FFA turnover rate (=uptake) might be utilized for calorigenesis by peripheral tissues before and during norepinephrine infusion. On the other hand, the regression coefficient of rectal temperature on FFA concentration was seemingly greater in cold-acclimated rats than in controls (Fig. 2), although significancy of correlation between these values in control rats was at less than the lowest significant level (r=0.378, $P \fallingdotseq 0.05$). These results seem to be consistent with the suggestion of HSIEH *et al.* (1966) that an increase in the capacity of system utilizing oxygen and substrates, especially plasma FFA, might be induced

in the course of cold acclimation. The systems are likely to be activated by norepinephrine. It might be also inferred that the specific change in FFA metabolism occurred during the course of cold-acclimation is observable in FFA uptake process rather than FFA oxidation by peripheral tissues because of the relationships as shown in Figs. 2 and 3. In this respect, the increase in the capacity of FFA uptake might be mainly produced in muscles of cold-acclimated rats, since eviscerated rats showed a similar reference as in intract rats that the regression coefficient between plasma FFA turnover rate and its concentration in cold-acclimated rats was greater than that in controls (Figs. 1 and 4).

A number of investigators reported that the administration of norepinephrine to cold-acclimated rats brought about a marked increase in oxygen consumption in association with a marked increase in body temperature as compared with the responses in controls. Although mechanisms regulating these effects at cellular level are still obscure, the thermogenic action of norepinephrine is thought to be related to FFA mobilization from adipose tissues. However, dissociation of catecholamine-induced calorigenesis from lipolysis and glycogenolysis was also demonstrated in dogs and rats (KENNEDY and ELLIS, 1969). MEJESNER and JANSKY (1971) have shown that the metabolism of gracillis muscle was directly stimulated by norepinephrine, and that the muscular metabolic response to norepinephrine was due neither to changes in blood flow nor to the amount of substrates released under physiological conditions into the blood. Even though there are receptors responding to norepinephrine in striated muscles, the role of FFA in nonshivering thermogenesis could not be diminished, since striated muscles utilize preferentially plasma FFA rather than intramuscular depot fat, glycogen and plasma glucose as energy source (ISSEKUTZ *et al.*, 1964; PAUL *et al.*, 1966). PAUL *et al.* (1966) observed in conscious dogs infused with ^{14}C-palmitate that the onset of exercise is associated with a sudden expenditure of energy at a very high rate which immediately increase not only substrate utilization but also its mobilization without necessarily altering FFA level. In addition, they found that at the same FFA level the rate of FFA uptake was significantly higher during exercise than at rest, although the rate of FFA uptake increased linearly with FFA concentration during rest and exercise. It was inferred that the release of norepinephrine which has been shown to be proportional to the metabolic rate during exercise undoubtedly play a significant role in substrate mobilization to support the new energy requirement. On the other hand, DIETERLE *et al.* (1973) found that lactic acid which is the major product from muscle during exercise stimulated FFA uptake and its oxidation in the muscle tissue *in vivo* and *in vitro*. Accordingly, they assumed that lactic acid might be a regulating factor for the increased FFA uptake during muscular exercise. However, in our previous study (MORIYA *et al.*, 1974) it was shown that blood lactate concentration was not altered during norepinephrine infusion in cold-acclimated rats under the same conditions as in this study.

PAUL and HOLMES (1973) reported that at the same FFA level, FFA turnover

rate was significantly lower in thyroidectomized dogs than in normal dogs, and that the amount of FFA oxidized was the same in these two groups when compared at the same level of FFA turnover rate. They concluded that the absence of thyroid hormone resulted in a suppression of FFA uptake as well as FFA release but not FFA oxidation. It was recognized that the thyroid hormone has an effect on the metabolic rate and plays an important role in the maintenance of body temperature. The importance of the hormone in the control of lipid metabolism was also demonstrated by DEBONS and SCHWARTZ (1961). There is evidence indicating that thyroxine is essential for the development of cold adaptation (LEBLANC and VILLEMAIRE, 1970; ITOH, 1974). The enhanced turnover rate of plasma FFA in cold-acclimated rats might be, at least in part, related to the increased secretion of thyroid hormone in animals exposed to cold.

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