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Q. THE ROLE OF CATECHOLAMINES IN COLD ADAPTATION

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INTRODUCTION

The accepted role of norepinephrine (NE) as a mediator at sympathetic nerve endings is the activation of smooth muscle causing vasoconstriction at arterioles. α-Receptors are postulated to accept the mediator and transduce the effect to muscular contraction. In classical physiology, epinephrine (E) is a mediator in increasing blood glucose levels and the levels of nonesterified fatty acids as well as in vasomotor effects. The sympathetic nervous system (19) and the adrenal medulla (6) play an integral role in the acute response to cold.

Studies in newborn animals exposed to cold and in cold-acclimated animals receiving a cold stimulus have broadened the role assigned to NE since it has a calorigenc effect under these circumstances and its effect on blood pressure and peripheral resistance is altered. The results are by no means unequivocal. The calorigenic effect of catecholamines is well demonstrated in mammals but not in birds. The biochemical mechanism of the effect in mammals may be postulated but the evidence is not definitive. The data concerning changes in peripheral circulation are equivocal. If it is accepted that peripheral resistance is lower after chronic exposure to cold, the mechanism still awaits explanation. By far the most exciting question is the shift in the control processes which activate nonshivering thermogenesis in preference to muscular shivering and alter the heat exchanger mechanism to provide for greater perfusion of the extremities with the conservation of heat.

METABOLIC EFFECTS

The reduction in shivering with continued exposure to cold was first described in cold-exposed rats (39). The nonshivering mechanism was studied in curarized rats (9), the mechanism was ascribed to NE (23), and the control was determined to be mediated *via* the sympathetic nervous system (24). Subsequently the response has been confirmed in the laboratory rat and described in wild rats (18), rabbits (29), dogs (35), cats (17), and man (28). It has not been possible to demonstrate this phenomenon in birds (15). All of these experiments have previously been reviewed in detail (7, 16).

A similar phenomenon has now been described in newborn animals: kittens, rabbits and rats (34); guinea pigs (4); and babies (30). The regulatory nonshivering thermogenesis present at birth in the guinea pig is blocked by hexamethionium or pronethalol. The regulatory nonshivering thermogenesis is gradually replaced by shivering thermogenesis as the animal grows (5). In a 1-day-old guinea pig, exposure to cold increases oxygen consumption, although little shivering occurs as evidenced by electromyograms (fig. 1, left). The re-

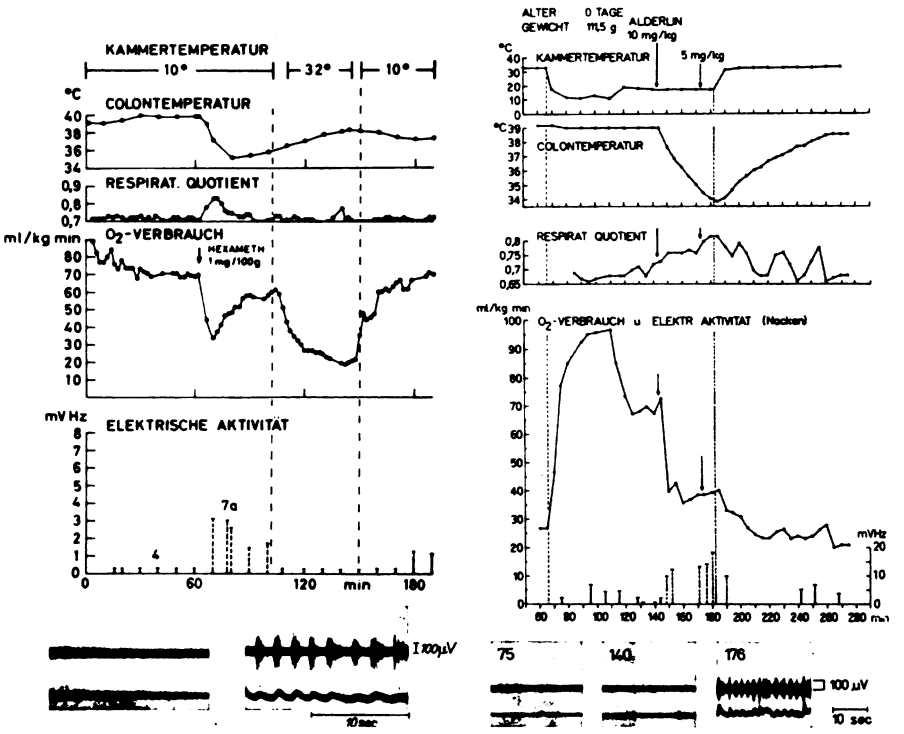

FIG. 1. Left, the influence of hexamethonium on a 1-day-old guinea pig. The colon temperature is maintained in a room of 10°C by increased oxygen consumption until the hexamethonium is injected. The falling colon temperature and oxygen consumption are accompanied by an increase in the electrical activity in muscle and an increase in the respiratory quotient. Right, influence of pronethalol (Alderlin) on the rise in oxygen consumption occasioned by exposure of a young (20 hr) guinea pig to a room temperature of 10°C. The decrease in oxygen consumption caused by pronethalol is accompanied by decrease in colon temperature, an increase in respiratory quotient, and an increase in muscular activity. (From Brück and Wünnenberg, Pflug. Arch. ges. Physiol. **282:** 376–389, 1965.)

spiratory quotient is near 0.70 during cold exposure and rises when the metabolic response is blocked by hexamethonium or pronethalol. Some shivering occurs when the autonomic blocking agent is used. As the animal matures this set of responses changes; chemical nonshivering thermogenesis is involved less, and there is greater activation of shivering (fig. 1, right).

Calorigenesis by NE decreases with age in rats kept at room temperature (fig. 2) (14). We may conclude that regulatory nonshivering thermogenesis is present to varying degrees in the newborn. As a regulatory mechanism this sympathetic mechanism is then replaced by motor nerve activity. Chronic cold exposure in the mature animal may reactivate the system present in the neonate.

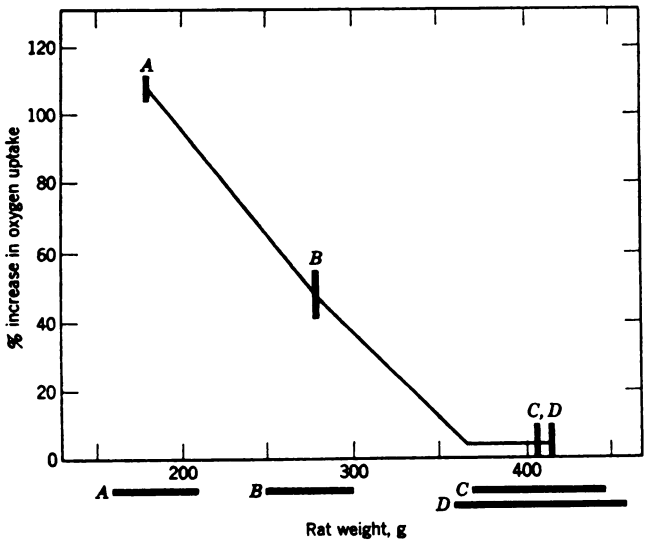

FIG. 2. The effect of age on the calorigenic effect of NE in the rat. The percent increase in oxygen uptake caused by NE is plotted against the weight of the rats. The weight of the rats is used as an indication of age. (From Himms-Hagen and Hagen, Ch. 11 in Actions of Hormones on Molecular Processes, ed. by Litwack and Kritchevsky, Wiley, New York, 1964.)

BLOOD LEVELS OF CATECHOLAMINES

Circulatory levels of NE, as deduced from urine output, are increased in the rat exposed to cold (17). In rats exposed to +3°C, NE increased to a maximum in the first 24 hr and fell to a level 2.5 to 3.5 times that of the control (+22°C) group. Levels of E are also elevated (fig. 3). There is an inverse relation between the fall in NE level and the development of sensitivity to the calorigenic effect

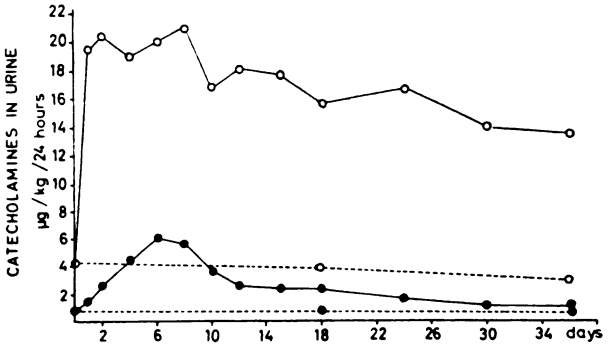

FIG. 3. Urinary excretion of E (solid circles) and NE (open circles) in rats weighing 170 to 180 g at +3°C (solid line) and +22°C (broken line). Each point represents the mean of 6 individual rats. (From Leduc, Acta physiol. scand. Suppl. 183, 1–101, 1961.)

of NE. The latter reaches full development after 24 days exposure to +6°C in rats (12).

ORGAN AND TISSUE EFFECTS

The calorigenic response to injected NE in the intact animal stimulated research into the origin or site of the increased metabolic activity at the tissue level. The first step in specifying the organs participating was taken by Depocas (11). Functionally eviscerated, curarized, cold-adapted rats show a large metabolic response to cold and to NE (13). This finding suggests a role for the muscles and negates the importance of the liver, a teleologically attractive site for the increased heat production. Jansky and Hart (26) measured the oxygen consumption of partly isolated leg muscles. During cold exposure and infusion of NE, oxygen consumption of the rat approximately doubled and the oxygen consumption of the leg muscle doubled without an increase in muscle blood flow. There was no increase in oxygen consumption of the kidney. The oxygen consumption of muscle increases without contraction on exposure of the animal to cold or with infusion of NE.

NE exerts a further role related to calorigenesis—the elevation of nonesterified fatty acids in the blood. This action suggests an effect on adipose tissue. The temperature of multilocular fat bodies in the newborn guinea pig changes with exposure to cold (3). This effect is blocked by pronethalol (fig. 4). NE stimulates the oxygen consumption and the release of free fatty acids from the brown fat of ground squirrels (27). Brown adipose tissue makes a significant contribution to the metabolic response of newborn rabbits to cold and NE (10). NE stimulates the oxygen consumption of rat epididymal adipose tissue from cold-acclimated rats in a manner quite different from that from warm-adapted rats (14). The effect of NE on the oxygen consumption of adipose tissue is enhanced by cold acclimation. The effect in the warm-acclimated rat is greatest in the young rat and diminishes with age (fig. 5). Thus the teleology that suggests the liver as the site for the effect of NE as an internal furnace suggests the fatty tissue as an insulating blanket. As circulating free fatty acids serve as a fuel for muscles, two effects (oxygen uptake of adipose tissue and increased muscle metabolism) may be coupled for enhancement of heat production. The increase in heat production with exposure to cold is accomplished with a respiratory quotient of 0.7 (see fig. 1). In warm-adapted rats, nonesterified fatty acids in the blood increase with increased concentration of infused NE (25), whereas in cold-adapted animals an increase in dose of infused NE is associated with a decrease in nonesterified fatty acids in the blood, the initial levels being higher (fig. 6, left). The calorigenic action of NE cannot be explained completely by the blood levels of fatty acids (fig. 6, right) as the oxygen consumption is inversely related to fatty acid concentration.

METABOLIC PATHWAY

The intermediary metabolism in cold acclimation has been reviewed by Smith and Hoijer (40) and Masoro (32). A characteristic of cold-induced nonshivering

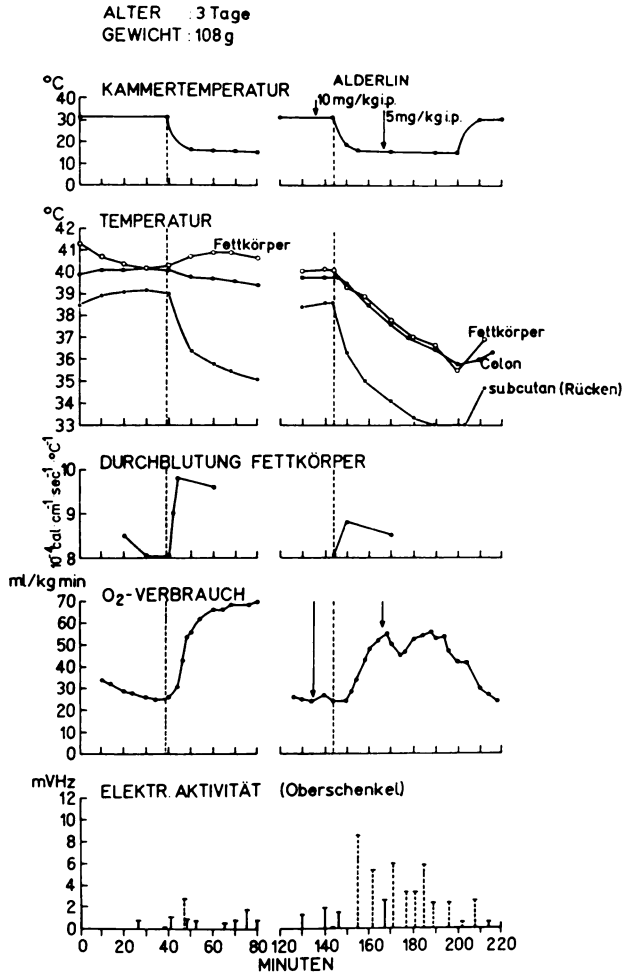

FIG. 4. The studies on a three-day-old unanesthetized guinea pig. As room temperature is decreased, the temperature of a fat pad increases, blood flow through the fat pad increases, and oxygen uptake of the animal increases. After pronethalol (Alderlin) the blood flow and temperature of the fat pad fall and the increased oxygen consumption of the whole animal is accompanied by an increase in the electrical activity of muscles. (From Brück and Wünnenberg, Pflug. Arch. ges. Physiol. **283**: 1–16, 1965.)

thermogenesis is the rapidity with which it is activated and inactivated. It is possible that normal biochemical procedures cannot determine the mechanism. Two general mechanisms are suggested: 1) an increased electron transport related to a decreased phosphorylative efficiency or 2) an increased ATP utilization without a rise in net work yield. Electron transport pathways of low phosphorylative efficiency (fig. 7) have been suggested by Potter (37). In this pathway TPNH-linked electron transport is the inefficient phosphorylative path (32). Beyer (2) demonstrated that tissues of cold-acclimated rats have an increased

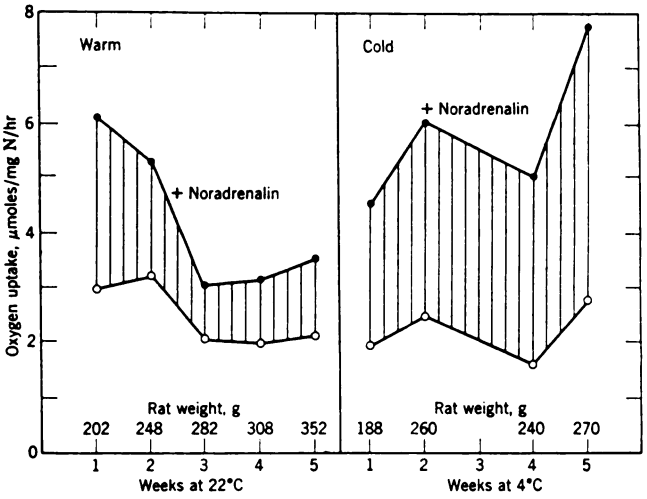

FIG. 5. Stimulation by NE of oxygen uptake of rat epididymal adipose tissue from warm-acclimated and cold-acclimated rats. The oxygen uptake, in micromoles per milligram of protein nitrogen per hour, of paired tissues—one incubated with (solid circles) and one without (open circles) NE, 10 micrograms per ml—is plotted against time. (From Himms-Hagen and Hagen, Ch. 11 in Actions of Hormones on Molecular Processes, ed. by Litwack and Kritchevsky, Wiley, New York, 1964.)

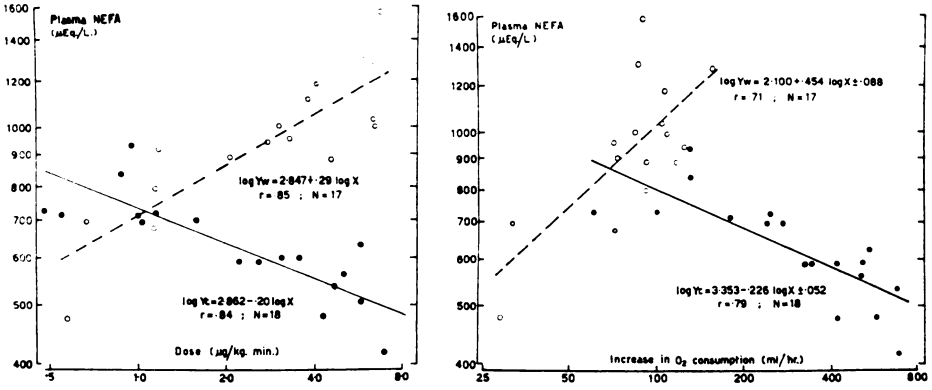

FIG. 6. Effect of infusions of NE for one-half hour in cold-adapted (solid circles) and warm-adapted (open circles) rats. (From Hsieh *et al.* (25).)

capacity for amobarbital-insensitive electron transport; this finding suggests that cold acclimation does activate pathways of low phosphorylative efficiency.

An increase in utilization of ATP might occur when the fatty acid synthesis-oxidation cycle operates in such a way that neither net synthesis or net oxidation takes place (fig. 8). The system becomes an ATP-utilizing, heat generator because the fatty acid synthesis requires a greater ATP input than is generated. Studies *in vitro* suggest this may occur (36). Ball and Jungas (1) suggested that an increase in the rate of the triglyceride lipolysis-fatty acid esterification cycle in

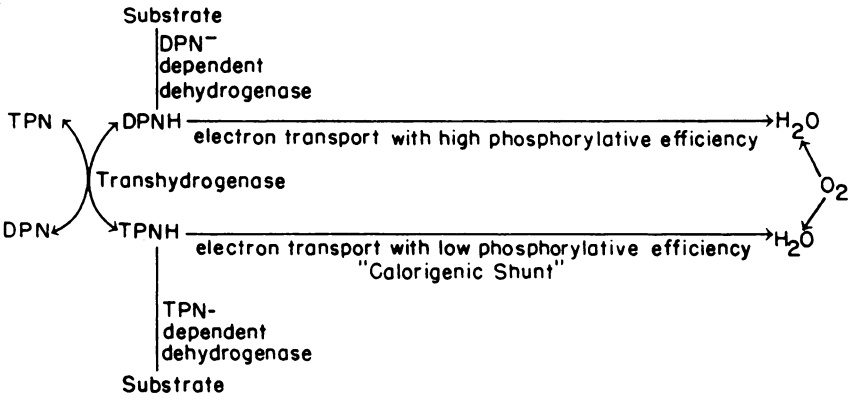

FIG. 7. A specific example of a potential calorigenic shunt that is a low phosphorylative efficiency electron transport pathway. (From Masoro, A consideration of energy and intermediary metabolism in relation to cold exposure. U.S.-Japan Cooperative Science Program, Seminar on Bioclimatology, Hokkaido University School of Medicine, Sapporo, Japan, November, 1964.)

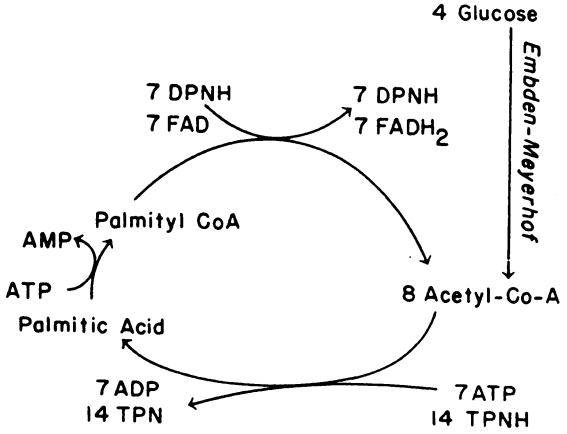

FIG. 8. Fatty acids synthesis-fatty acid oxidation cycle. The net potential ATP input during synthesis equals 49 moles per mole of palmitic acid formed. The net ATP yield upon the oxidation of 1 mole of palmitic acid to CO_2 and water equals 33 moles. (From Masoro, A consideration of energy and intermediary metabolism in relation to cold exposure. U.S.-Japan Cooperative Science Program, Seminar on Bioclimatology, Hokkaido University School of Medicine, Sapporo, Japan, November, 1964.)

adipose tissue may be involved in regulatory nonshivering thermogenesis (fig. 9). This system uses ATP and generates heat. The biochemical mechanisms activated by NE in regulatory nonshivering thermogenesis have yet to be clearly established.

CIRCULATORY EFFECTS

Animals chronically exposed to cold develop the capability to maintain the extremities above freezing temperatures. In man there is, with one exception,

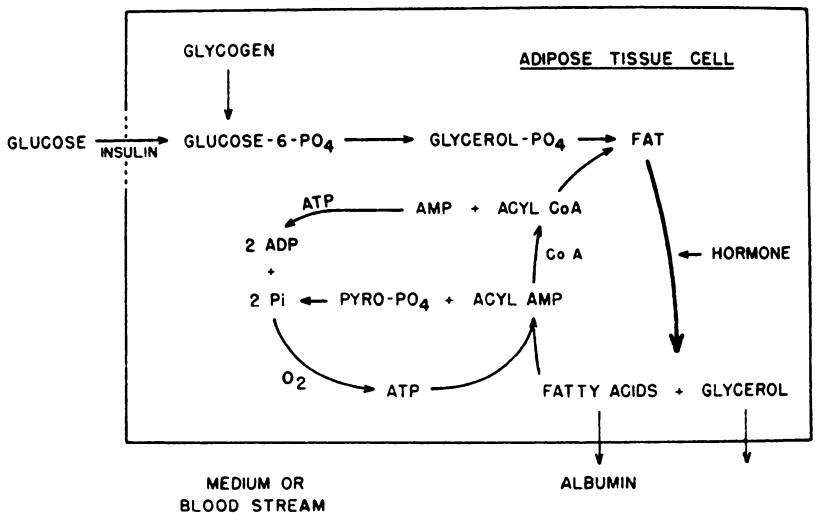

FIG. 9. A schema offered by Ball and Jungas (1) to account for the concomitant stimulation of oxygen consumption and fatty acid release in adipose tissue by certain hormones. (From Ball and Jungas, Proc. nat. Acad. Sci. Wash. 47: 932–941, 1961.)

an increase in skin temperature and blood flow following cold exposure (8). Prolonged exposure to cold causes rabbits to respond to intravenous infusion of NE or E at room temperature of 30°C with less decrease in ear temperature during infusion and more rapid return to control levels following infusion, less increase in peripheral resistance, and less effect on compliance of the veins (21) (fig. 10). Direct infusion of NE in saline in the rabbit ear at a constant flow rate in warm-adapted and cold-adapted rabbits has been accomplished at 10°C, 20°C and 30°C room temperature. The factor by which the perfusion pressure was increased by NE was considered an indicator of the vascular sensitivity to this substance. The sensitivity of the vascular bed was higher in the warm-adapted than in the cold-adapted animals at 30°C. At 20°C the difference was negligible and at 10°C the cold-adapted vascular bed showed a slightly higher sensitivity than the warm-adapted (38) (fig. 11).

An analysis of ear skin temperature and blood flow suggest a change in character of circulation to the ear. Honda (20) showed that in cold-acclimation the animal shifts circulation to increase heat exchange and increases the extent to which the core is involved in body cooling.

After prolonged exposure to cold, rabbits responded to infusion of E and NE with less change in heart rate during infusion and a more rapid return to control levels following infusion (fig. 10). The blood pressure response to infused *l*-norepinephrine is less in cold-adapted rats than in warm-adapted at low dosage (0.078 to 0.312 μg/kg) but the difference disappears at a higher dosage (0.625 to 2.5 μg/kg) (25). These data on peripheral resistance and heart rate may be explained by a reduced sensitivity to NE or an increased rate of its destruction. The higher blood levels suggest the former. The extent to which blood flow to the periphery

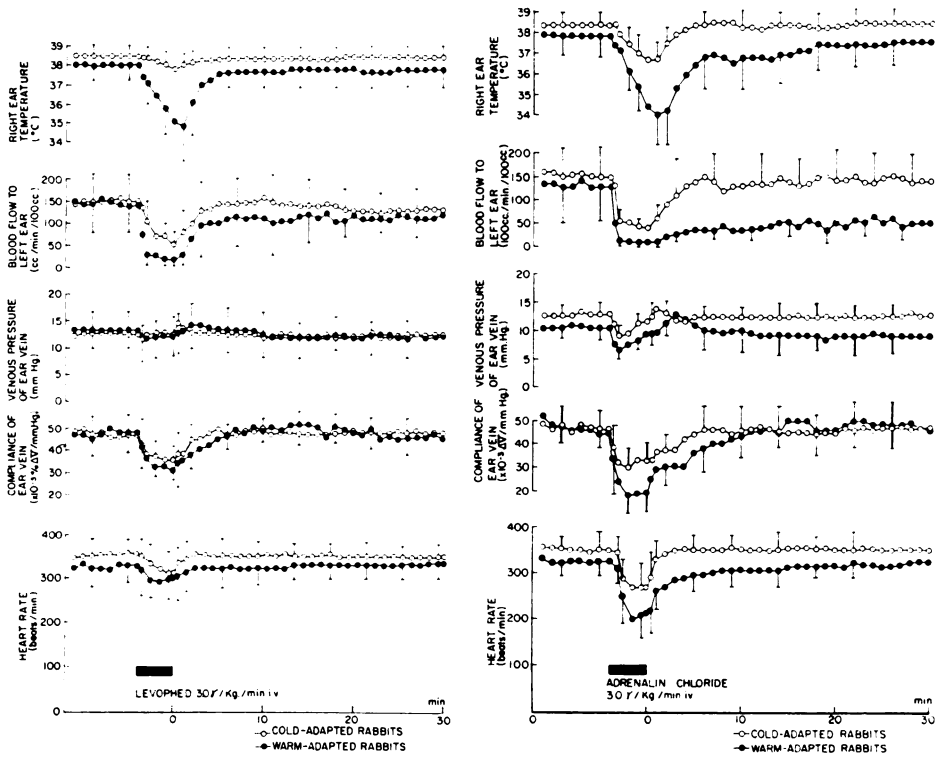

FIG. 10. The effects of NE (left) or E (right) on ear temperature, blood flow to the ear, venous pressure, vein compliance and heart rate in cold adapted and warm adapted rabbits. (From Honda *et al.*, J. appl. Physiol. **17**: 754–758, 1962.)

changes with temperature of the extremities depends on the body heat content. Thus the peripheral effect of NE in systemic injection may be secondary to the change in heat production. The dosage levels in the experiments and the direct infusion experiments suggest that the circulatory effect is distinct from the metabolic effect.

Although this summary of effects is limited to catecholamines in cold acclimation, the interrelation of hormones is an inescapable fact in the overall reactions. The most significant interaction is with thyroxin. Swanson (41) reported the difference in metabolic response to an injected dose of E in cold-acclimated and warm-acclimated animals on the titration curve of injected thyroxin. There are many similarities between hyperthyroid animals and cold-acclimated animals. Thyroxin turnover is increased in the cold-acclimated rat; the thyroxin requirement to maintain growth and metabolism is doubled although animals can survive with minimal thyroxin levels; and TSH levels are high. The rise of plasma free fatty acids in response to injected E or NE and the hypoglycemic effect of insulin are increased. Hsieh (22) concluded, however, that rats exposed to cold require more thyroid hormone and may be suffering from a thyroid deficiency in the cold.

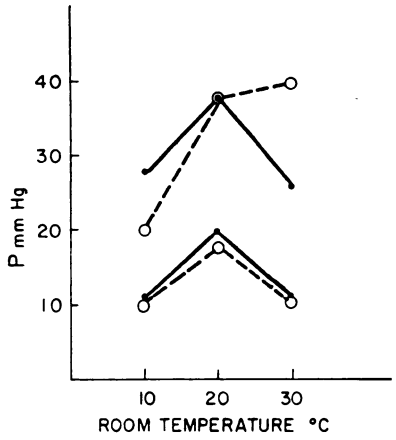

FIG. 11. The influence of temperature and the infusion of NE on the infusion pressure at a constant flow rate in the rabbit ear. The closed circles are cold-adapted rabbits; the open circles are warm-adapted rabbits. The infusion pressure is given on the ordinates at different temperatures of infusion fluid (abscissae). The lower curves are Tyrode-Ringers; the upper curves are Tyrode-Ringers plus NE, 200 μg/ml. Tyrode-Ringers was infused at a constant rate, 4.5 ml/min through the central artery at the base of the ear and collected from the central vein. (From Reite *et al.* (38).)

New techniques of chemostimulation have implicated catecholamine levels in the brain in behavioral responses (33). The activation of aspects of the homeostatic system may also be a role of catecholamines in cold adaptation.

In conclusion, the studies of catecholamine responses in chronic exposure to cold unfold fascinating questions from the biochemical to the control systems level. There are ontogenetic and phylogenetic aspects of the problem.

Activation of the regulatory nonshivering thermogenesis by exposure to cold makes use of sympathetic pathways. A thermal benefit is accompanied by a circulatory shift to maximize the role of the body shell in insulation. Possible metabolic pathways exist and peripheral (nonvisceral) adipose tissue may serve as the site of heat production although mechanisms within muscle tissue may not be excluded.

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