(Table II), in good agreement with published data obtained at higher pH values[9,10]. This is significantly larger than the maximum value for the association of pepsin with native ovalbumin.

The temperature-dependence of K_m (Table I) yields values for the changes in normal enthalpy and entropy that are not significantly different for the 2 forms of the substrate. The large errors inherent in the method used for obtaining those values do not allow a fine interpretation, but it is significant that the association of pepsin and ovalbumin is an endothermic process with a large increase in entropy. This also occurs in the peptic hydrolysis of small synthetic substrates[11], and would indicate that hydrophobic bonding plays a preponderant role in the association of pepsin with its substrate.

Table II. Effect of temperature on the rate of acid denaturation of ovalbumin

Temperature (°C)	25.0	30.0	35.0	40.0
$k_1 \times 10^3$ (min^{-1})	7	20	55	130

The first-order rate constants (k_1) were estimated by following the decrease of solubility of $2.5 \times 10^{-4} M$ ovalbumin solutions kept at pH 0.8. The solubility was determined by periodically removing aliquots that were diluted 40-folt with $2 M$ acetate buffer (pH 4.75) containing $0.5 M$ $MgSO_4$, and reading the absorbance of the filtrate at 275 nm.

Résumé. Partant de l'effet de la température sur la cinétique de la protéolyse peptique de l'ovalbumine native ou denaturée, on a estimé l'énergie d'activation, l'enthalpie et l'entropie de l'association enzyme-substrat.

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Norepinephrine-Sensitive Na^+/K^+ ATPase Activity in Brown Adipose Tissue[1]

Norepinephrine (NE) released from sympathetic neuronal terminals appears to mediate the increased thermogenesis in brown adipose tissue during cold stress[2,3]. This response, which is blocked by β-adrenergic antagonists, apparently involves activation of lipolysis via the adenyl cyclase, 3′,5′-cyclic AMP system[4,5]. However, the biochemical mechanisms underlying the NE-stimulation of respiration in brown fat are still controversial[3].

The uncoupling agent, 2,4-dinitrophenol (DNP), injected i.v. into cold-acclimated rats, enhances the thermogenic response of the brown fat during cold stress as well as during NE administration; this implies that in activated brown fat, respiration is coupled to oxidative phosphorylation[6]. We thus proposed a) that the NE-induced respiratory elevation of the brown fat initially reflects increased availability of substrate rather than of ADP, and b) that this is accompanied by an increased cellular ATP requirement[6].

The finding that NE (whether of exogenous or neuronal origin) depolarized the membranes of these cells in vivo[7], suggested that in the Na^+/K^+ distribution and perhaps also the Na^+/K^+ pump, alterations might occur during stimulation of thermogenesis by the catecholamine. Hence we examined the effect of NE on the Na^+/K^+ ATPase system associated with the membrane ion pump.

The methodology entailed removal of brown adipose tissue from decapitated male, Long-Evans rats that had been cold acclimated (exposure to 5 °C, 50% R.H., with 12 h high/low light cycle for 4–8 weeks). The brown fat was cleared of extraneous tissues and then homogenized in a mixture (9/1, volume/weight) containing 250 mM sucrose, 2 mM EDTA, and 2 mM TES (N *Tris* (hydroxymethyl) methyl-2-aminomethane sulfonic acid), pH 7.0 at 0 °C. The homogenate was centrifuged 10 min at $14{,}000 \times g$, the overlying fat removed and the supernatant decanted for assay. ATPase activity was determined in 2 media (final volume 1.5 ml): i.e., (A) in millimoles per liter: 20 TES (pH 7.2), 27 sucrose, 8.7 KCl, 70 NaCl, 2.7 EDTA, 5 $MgCl_2$, 4 NaCN, 6.7 Na_2ATP (Sigma); (B) differed from (A) only in containing 190 mM sucrose and no KCl or NaCl. Each reaction system contained 0.477 ± 0.013 mg supernatant nitrogen[8]. After incubation at 30 °C for 20 min, the reaction was terminated with 0.5 ml $1.0 N$ $HClO_4$, the tubes centrifuged at 0 °C for 10 min at $1000 \times g$ and the inorganic phosphate in the supernatant assayed[9] against appropriate controls. The Na^+/K^+ ATPase activity is here defined as the inorganic phosphate released in the presence of Na^+, K^+, and Mg^{++} (medium A) minus that in the absence of KCl and NaCl (medium B)[10].

In the presence of NE, the Na^+/K^+ ATPase was markedly stimulated. The increase was dose dependent (Figure) and maximal with 6 mM NE. The fact that this enhancement was abolished by ouabain (Table) suggests that this ATPase is part of the Na^+/K^+ pump associated with the cell membrane.

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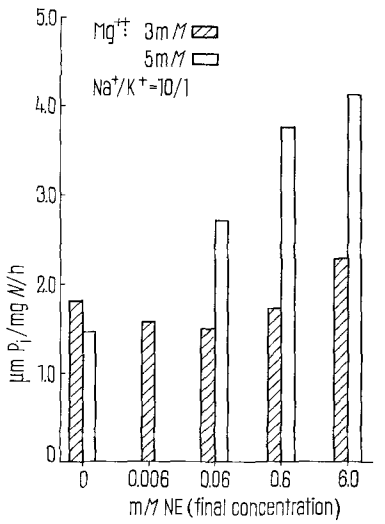

Effect of varying concentrations of norepinephrine (NE) on the Na^+/K^+ ATPase of brown fat.

Effect of ouabain and adrenergic blockers on norepinephrine stimulation of Na^+/K^+ ATPase of brown fat[a]

Final Concentration (mM)	Inhibition of NE (6 mM) Stimulation (%) Ouabain (N)	Propranolol (N)	Phentolamine (N)
0.0004	–	1.3 (2)	–
0.004	–	100 (1)	–
0.010	49 ± 18 (5)	–	–
0.040	–	97 ± 15 (4)	50 ± 14 (4)
0.10	96 ± 16 (7)	–	–
0.40	–	98 (2)	80 (3)
0.80	–	100 (1)	73 (3)
1.0	104 (2)	–	102 (1)
2.0	–	–	100 (1)

[a] Values = mean ± S.E.; (N) = number of trials; 6 mM NE stimulated the Na^+/K^+ ATPase 97.9 ± 11.5% ($N = 12$; mean activity with 6 mM NE = 4.507 ± 0.106 µmoles phosphate hydrolyzed/mg nitrogen/h).

In addition, the response to NE appears to be at least 100 times more sensitive to the β-adrenergic antagonist, propranolol, than to the α-blocker, phentolamine (Table). Thus, the NE-stimulation of the ouabain-sensitive ATPase can probably be considered as a β response and therefore may be associated with the adenyl cyclase system. We find however that theophylline, added over a wide range of concentrations (2 µM to 2 mM), neither significantly stimulates nor does it act synergistically with added NE, suggesting that the NE enhancement of ATPase activity is independent of cyclic AMP. Therefore, if the response of the ATPase activity to NE is related to the β-adrenergic pathway as presently defined in brown fat (i.e., the adenyl cyclase, cyclic AMP system), it must be associated with events occurring prior to formation of the cyclic nucleotide; alternatively, this ATPase stimulation represents a second β response of the tissue.

The present study thus demonstrates that addition of NE in vitro enhances the activity of an ouabain-sensitive Na^+/K^+ ATPase. Moreover, this stimulation is antagonized by low doses of the β-blocker, propranolol. Although the Na^+/K^+ pump of the cell membrane appears to be altered, the relationship between this phenomenon and that of the NE-induced membrane depolarization is not yet clear. However, the existence of a NE-sensitive Na^+/K^+ ATPase is consistent with our previous proposal that NE induces an altered state in the brown fat in which the elevated rate of oxygen consumption (heat production) is accompanied by increased ATP turnover.

Zusammenfassung. Norepinephrin fördert in vitro die Tätigkeit einer Ouabain empfindlichen Na^+/K^+ ATPase. Diese Wirkung, durch eine geringe Dosis eines β-adrenergischen Antagonisten eingeschränkt, scheint offenbar von einer 3', 5'-zyklischen AMP unabhängig zu sein. Die Tätigkeit der Brenzkatechin-induzierten ATPase stimmt mit der Auffassung überein, dass im aktivierten braunen Fettgewebe der Energieumsatz zusammen mit dem vermehrten Sauerstoffverbrauch ansteigt.

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Effect of Naphthoquinone Pigment, Xanthomegnin from *Microsporum cookei* on the Respiration of Rat Liver Mitochondria

It is well known that mycelia of many species of dermatophytes produce pigments[1-16]. Recently, using thin-layer and column chromatography, several pigments such as aurosporin, xanthomegnin, violosporin, citrosporin, rubrosporin, luteosporin and iridosporin[15] were isolated by us from *Microsprum cookei* HUT-2061, a fungus which shows to be analogous quinone or lactone compounds. Xanthomegnin (Figure 1) is the most abundant of these pigments and its chemical structure has been shown by BLANK and JUST[13] to be 3, 3'-*bis*[2-methoxy-7-(2-hydroxy propyl)-8-carboxy-1, 4 naphthoquinone lactone].

Since little attention has, however, been paid toward the elucidation of biochemical significances of these pigments, we performed some preliminary experiments to determine effect on the respiratory system of the isolated rat liver mitochondria.

Material and methods. The fungus *Microsporum cookei* HUT-2061 was kindly supplied by Dr. HASEGAWA, Department of Veterinary Internal Medicine, Tokyo University.

Xanthomegnin was isolated and purified by the method described previously[12], and an acetone solution was used in this study. The final concentration of acetone in the reaction mixture did not exceed 2%. Sodium adenosine-5'-diphosphate (ADP) and bovine serum albumin were purchased from Sigma Co.

Rat liver mitochondria were prepared according the method of SCHNEIDER[17] with a slight modification using a solution containing 0.25 M sucrose, 0.2 mM EDTA and