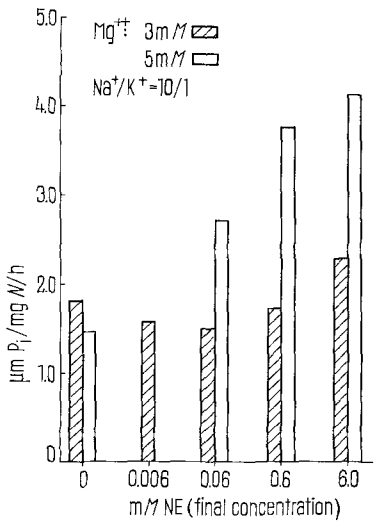

Effect of varying concentrations of norepinephrine (NE) on the Na^+/K^+ ATPase of brown fat.

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

2.5 m*M* *Tris*-HCl (pH 7.0). The amount of mitochondrial protein was estimated by the biuret method using bovine serum albumin as standard.

Mitochondrial respiration was examined polarographically at 25° with the Clark-type electrode (Beckmann). The *P/O* (ADP/*O*) ratio and respiratory control ratio were calculated graphically by the method of CHANCE and WILLIAMS[18] by adding a known amount of ADP measured at 260 nm.

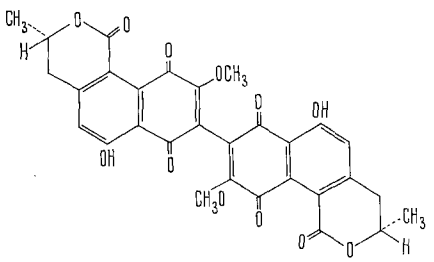

Fig. 1. Chemical structure of xanthomegnin.

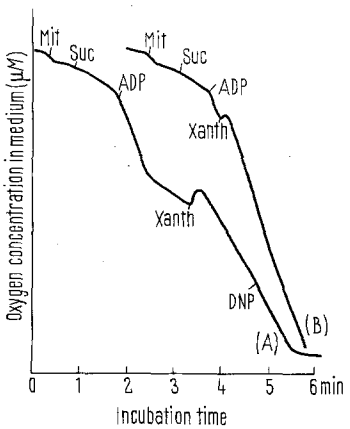

Fig. 2. Effect of xanthomegnin upon mitochondrial respiration. The standard reaction medium was an air-equilibrated solution containing 0.3 *M* mannitol, 10 m*M* KCl, 2.5 m*M* $MgCl_2$ 10 m*M* potassium phosphate, 0.2 m*M* EDTA and 10 m*M* *Tris*-HCl in a final volume of 4.5 ml (pH 7.4). The following additions were made: 0.3 ml of mitochondrial suspension (6 mg mitochondrial protein); 0.05 ml of 0.5 *M* succinate; 0.05 ml of 20 m*M* ADP; 0.05 ml of 10^{-8} *M* xanthomegnin; 0.05 ml of 5×10^{-4} *M* DNP. Preincubation was carried out and the reaction was initiated by addition of succinate at 25°. Xanth, xanthomegnin; Mit, mitochondria; Suc, succinate; DNP, 2,4 dinitrophenol; ADP, 2Na-adenosine 5′-diphosphate.

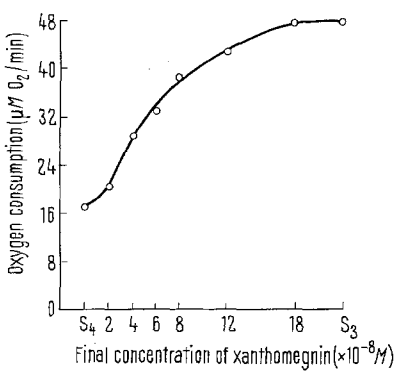

Fig. 3. Influence of the concentration of xanthomegnin. The composition of the standard reaction mixture is the same as in Figure 2. S_3, state 3; S_4, state 4.

The standard reaction medium used in these experiments consisted of 0.3 *M* mannitol, 10 m*M* KCl, 2.5 m*M* $MgCl_2$, 10 m*M* potassium phosphate, 0.2 m*M* EDTA and 10 m*M* *Tris*-HCl (pH 7.4), its total volume being 3.5 ml.

Results and discussion. The present mitochondria isolated from rat liver showed respiratory control and *P/O* ratios, 4.76 and 1.74, respectively.

Typical polarographic traces of oxygen consumption by the mitochondria utilizing succinate as a substrate are shown in Figure 2. Addition of ADP to the reaction mixture produced a characteristic acceleration in the respiration (state 3 as defined by CHANCE et al.[18]), and this higher respiration continued until all the added ADP was completely depleted (state 4). Further, the addition of quinone compound, xanthomegnin to the state 4 respiration caused a marked acceleration in the respiration (curve A), while no effect on respiration was observed rate when xanthomegnin was added to state 3 respiration (curve B). This therefore indicates that xanthomegnin has an uncoupling effect on mitochondrial respiration.

The influence of the concentration of this compound on respiratory control is illustrated in Figure 3. It is evident that xanthomegnin can act as uncoupler even at a concentrations as low as 10^{-7}–10^{-8} *M*.

In summary, it is worthy to note that a 1,4 naphthoquinone pigment, xanthomegnin, isolated from a pathogenic fungus, *Microsporum cookei* showed the marked uncoupling in the oxidative phosphorylation of the isolated mitochondria from rat liver.

Preliminary studies on the other pigments mentioned above indicated that they also may act as uncouplers much like xanthomegnin.

Résumé. Un pigment naphtoquinonique extrait de *Microsporum cookei* présenta une forte action découplante dans la phosphorylation oxydative des mitochondries du foie de rat.

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