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## Uncoupling of Oxidative Phosphorylation by Ultrafiltrates Of Uremic Serum. (32040)

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Previous investigation has suggested that one or more substances toxic to tissues are present in the serum of individuals suffering from uremia(1-5). Recently, Bittar (personal communication) has shown that an ultrafiltrate of serum from uremic patients will inhibit the sodium, potassium adenosinetriphosphatase (ATPase) of crab muscle. Renner and Heintz(5) reported aberrations of metabolism in kidney cortex in the presence of factors from serum of uremic individuals, and the respiration of isolated mitochondria was thought to be uncoupled in the presence of certain protein free fractions isolated from such sera. This report presents data indicating that an ultrafiltrate of sera from uremic persons does indeed produce a partial uncoupling, and further that this uncoupling appears to be associated only with adenosinetriphosphate (ATP) production associated with electron transport between reduced nicotinamide-adenine dinucleotide (NADH) and cytochrome *b* (Site I).

*Materials and methods.* All studies were carried out on rat liver mitochondria isolated by the Schneider and Hogeboom technique from a 10% (w/v) 0.25 M sucrose homogenate of rat liver(6). The twice washed mitochondria were suspended at a concentration of 50 mg protein/ml in 0.25 M sucrose before use. The respiration of the isolated mitochondria was studied using a Clark oxygen electrode connected to appropriate amplifiers and recorders(7).

Normal and uremic serum ultrafiltrates were obtained by centrifugation through a cellophane membrane at 4°C or by use of a Schleicher and Schuell vacuum ultrafiltration apparatus which utilizes a collodion membrane.

All patients whose sera were used had symptoms of renal failure and had blood urea nitrogen (BUN) levels of 100-300 mg % (average of 177 mg %) and serum creatinine levels of 5.4-30 mg % (average of 15 mg %). Of the 7 patients with chronic renal failure, 3 patients had diagnoses of nephrosclerosis, 3 patients had chronic pyelonephritis, and one had glomerulonephritis. Two patients had acute renal failure secondary to pneumonia and surgery. Several patients were receiving medication including

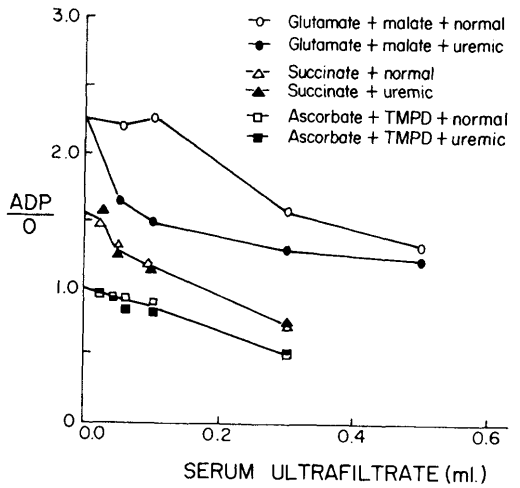


FIG. 1. Uncoupling of oxidative phosphorylation by normal and uremic serum ultrafiltrates. The control reaction medium contained in a final volume of 2.0 ml, 50 mM Tris-HCl, 50 mM KCl, 5 mM $MgNO_3$, 2.5 mM $K_2HPO_4$, 0.5 mM EDTA, 2.5 mg/ml defatted bovine serum albumin, 5-6 of mitochondrial protein and, where indicated, 5 mM glutamate plus 5 mM malate, 10 mM succinate, or 10 mM ascorbate plus 0.3 mM tetramethyl-p-phenylenediamine. The pH was 7.5 and temperature 25°C. In those tubes which contain serum ultrafiltrates, KCl concentration was decreased by an amount necessary to compensate for the number of osmoles of added ultrafiltrate. The ADP/O ratio was estimated in the tightly coupled mitochondria by analysis of the polarographic traces after addition of known amounts of ADP as described by Chance and Williams(8).

digoxin, penicillin, cephalothin, methyl dihydroxyphenylalanine, and thiazide diuretics. The sera of all of these patients behaved identically in the assays to be described below.

*Results and discussion.* The effect of the addition of increasing amounts of normal and uremic serum ultrafiltrates to the mitochondria are shown in Fig. 1. It can be seen that with a mixture of glutamate and malate as substrate there is a decrease in ADP/O ratio at a much lower concentration of uremic ultrafiltrate than there is with the addition of normal serum ultrafiltrate. Also, it should be noted that this uncoupling plateaus off after a decrease of about 1.0 ADP/O unit. This effect has been noted in all 11 of the uremic patients studied thus far. A similar effect is seen with other NADH linked substrates such as beta-hydroxybutyrate and pyruvate.

When succinate was used as a substrate, no difference was noted in the effects of normal or uremic serum. Also, when ascorbate and N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD) are used as substrate for mitochondrial oxidation, no difference was seen in the effect of normal and uremic serum ultrafiltrate.

Oxidative phosphorylation coupled to succinate oxidation involves only two sites of phosphorylation between cytochrome *b* and oxygen, and the phosphorylation associated with ascorbate oxidation involves only a single site between cytochrome *c* and oxygen. Thus, it would seem that this "uremic" uncoupling occurs only at the phosphorylation site associated with the oxidation of NADH and the reduction of cytochrome *b* (Site I), since this site is not involved in the oxidation of either succinate or ascorbate by the mitochondria.

It is possible, however, that a true uncoupling has not occurred. Quinones, such as vit $K_3$, (menadione) have been shown by Ernster and coworkers(9-11) to shunt electrons around the rotenone and amytal sensitive site in the respiratory chain. When menadione and rotenone are added to mitochondria, as Ernster(11) reported, the ADP/O ratio is reduced approximately one unit. The NADH linked phosphorylation site has been shunted by way of the DT diaphorase(9,10) and electrons reenter the chain, probably at the level of cytochrome *b*. Also, the rotenone sensitive site is probably identical with the site of interaction of the phosphorylative pathway and the respiratory chain between NADH and cytochrome *b*. If the substance present in uremic serum does shunt around this portion of the electron transport chain like the quinones, then respiration in the presence of uremic serum should not be sensitive to rotenone. In the presence of rotenone and menadione, when the electron flow is shunted around phosphorylation Site I and then back into the normal pathway of electron flow at the level of cytochrome *b*, the subsequent addition of uremic filtrate should cause no uncoupling. Such an artificial system, with a NADH linked substrate as a source of electrons has only the two functioning phos-

TABLE I. Effect of Rotenone and Menadione on Uncoupling by Serum Ultrafiltrates. The reaction medium and conditions are the same as in the Figure except that the substrate is 10 mM beta-hydroxybutyrate. 0.1 ml of uremic and normal serum ultrafiltrates were added where indicated and rotenone where added was 0.5 $\mu$M and menadione 10.0 $\mu$M.

| Additions | State 3 respiration (m$\mu$ at. O/min) | ADP/O |
|---|---|---|
| None | 279 | 2.6 |
| + normal filtrate | 277 | 2.5 |
| + uremic " | 279 | 2.0 |
| + rotenone | 25 | — |
| + normal filtrate + rotenone | 25 | — |
| + uremic filtrate + rotenone | 25 | — |
| + rotenone + $K_3$ | 426 | 1.4 |
| + normal filtrate + rotenone + $K_3$ | 418 | 1.4 |
| + uremic filtrate + rotenone + $K_3$ | 438 | 1.4 |

phorylation sites, those between cytochrome *b* and oxygen.

The results of such an experiment are shown in Table I. In the control experiment, uremic serum ultrafiltrate uncouples about 0.6 of an ADP/O unit. It should also be noted that there is no effect of normal or uremic ultrafiltrate on rate of respiration. 0.5 $\mu$M rotenone inhibits mitochondrial respiration in the presence of substrate, oxygen, phosphate and ADP (state 3) by 91% in the presence or absence of either uremic or normal ultrafiltrate. Thus, the uremic ultrafiltrate does not shunt the flow of electrons around phosphorylation Site I as menadione would. The further addition of normal or uremic ultrafiltrate to this system does not result in any further effect on either the respiration rate, in the presence of substrate, oxygen, phosphate and ADP, or on the ADP/O ratio.

Thus, the substance or substances present in the serum or ultrafiltrate of uremic persons does indeed uncouple oxidative phosphorylation of rat liver mitochondria and the uncoupling occurs only at Site I. This effect is compatible with the reported inhibition of sodium, potassium dependent ATPase in the *Maia* muscle fiber and with the metabolic changes noted by Renner and Heintz (5) in kidney cortex slices.

The uncoupling produced by ultrafiltrates is also seen when whole sera are used. Preliminary attempts to purify the uncoupling substance indicate that it is cationic at pH 8.0 and soluble in acidic ether.

The relevance of the above studies to the metabolic state of the tissues of uremic individuals is unknown. It will be necessary to ascertain whether or not mitochondria from tissues of uremic individuals are uncoupled in this specific manner. Also, the nature of this uncoupling material should be determined. Studies toward these ends are under way.

*Summary.* The influence of substrates in serum ultrafiltrates of uremic individuals on isolated rat liver mitochondria has been determined. A substance or substances present in such an ultrafiltrate does uncouple oxidative phosphorylation, but only at the phosphorylation site linked to the respiratory chain between NADH and cytochrome *b*. The inhibiting substance is cationic at pH 8.0 and is soluble in acidic ether.

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