OXYGEN CONSUMPTION AND UTILIZATION OF CARBOHYDRATE AND 
FAT METABOLITES IN KIDNEY CORTEX SLICES AND IN 
BRAIN HOMOGENATE USING SERA OF CHRONIC URAEMIC PATIENTS*

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The pathway of intoxication in patients suffering from chronic uraemia is, in spite of a 
umitidue of hypotheses, still an unsolved problem. In many cases disturbances of acid-
base or water metabolism are predominant. Often, however, there is clinical evidence for 
substances coming into being or being retained in chronic renal failure which may induce 
uraemic intoxication. The presumption that toxic compounds accumulate in uraemia is 
supported by the efficacy of haemodialysis as well as the findings of Henkin et al. (1961), who 
isolated substances toxic to HeLa cells from bath fluid of an artificial kidney. Based on the 
finding of a raised acetoin level in the blood of chronic uraemic patients, Thölen, Bigler, and 
Straub (1962) suggested an alteration of pyruvate utilization. Attempting to clarify this 
problem experimentally, we investigated respiration and some aspects of metabolism of kid-
ney and brain tissue in sera of chronic uraemic patients.

Materials and methods

Incubation and measurement of respiration of rat kidney cortex slices and of rat brain 
homogenate was performed in a Warburg apparatus, using the ‘one vessel method’ with 
separated carbonate-bicarbonate buffers and constant CO₂ pressure. The vessel was that of 
Dickens and Simer (1931), the outer ring containing the carbonate-bicarbonate mixture, and 
the central compartment containing incubation medium and tissue. Sera of patients suffering 
from chronic uraemia (NPN above 200 mg/dl) were used as incubation media. Control 
measurements were carried out using sera of patients suffering from acute uraemia (acute 
anuria due to shock, haemolysis, etc.), from other medical diseases (duodenal ulcer, pneu-
monia, heart failure, carcinoma, bronchial asthma) and of healthy persons. All sera were 
diluted 1 : 1 with Krebs-Ringer bicarbonate solution.

After addition of various substrates (pyruvate, D, L-β-hydroxybutyrate, acetoacetate, 
glucose), their utilization and, in the cases of pyruvate addition, the formation of glucose 
were measured. Pyruvate was determined according to Bücher et al. (1962), D(--)-β-hydroxy-
butyrate and acetoacetate according to Krebs et al. (1962), hydroxybutyrate racemate 
according to Lester and Greenberg (1948), amino acids according to Schlayer (based on Van 
Slyke’s ninhydrin method), lactate according to Hohorst (1962), and glucose with the 
glucose-oxidase method. Acetoacetate was prepared according to Ljunggren (1924), the content 
of its stock solution being measured with Edson’s (1935) anilin citrate method. Mitochondria 
were prepared according to Weinbach (1961). The pH of all incubation media was between 
7.35 and 7.44.

Results

Using the sera of patients suffering from chronic uraemia, we found kidney cortex slices to 
utilize less pyruvate or L(+-)-β-hydroxybutyrate or acetoacetate than slices incubated under

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the same conditions in control sera and in sera of patients suffering from acute uraemia. Furthermore, the formation of glucose from pyruvate was lowered. The consumption of D(--)-β-hydroxybutyrate remained unchanged or was slightly increased. The oxygen consumption remained the same. Figure 1 shows the results using five uraemic and ten control sera. Except for consumption of oxygen and D(--)-β-hydroxybutyrate, the differences were highly significant (p < 0.01). Figure 1 shows furthermore the results using brain homogenate which was incubated in the same sera as above. The utilization of glucose and pyruvate is decreased, the consumption of amino acids, however, is increased. The oxygen consumption remained the same.

![Graph](image)

**Fig. 1.** Consumption of oxygen, D,L-β-hydroxybutyrate, L(+)β-hydroxybutyrate, D(--)-β-hydroxybutyrate, pyruvate and acetocetate. L(+)β-hydroxybutyrate calculated as difference between D,L-β-hydroxybutyrate and D(--)-β-hydroxybutyrate.

Mean values and S.D.

In an attempt to isolate toxic compounds, uraemic sera were passed through a column of Sephadex G25. One of the protein-free fractions (‘fraction III’) containing among others ninhydrin-positive substances exhibited an uncoupling of oxidative phosphorylation by mitochondria (Table 1). Analogous fractions from the control sera had no effect on oxidative phosphorylation. The degree of toxicity depends upon the amount added. Finally, Figure 2 shows the metabolic activity of kidney cortex slices and of brain homogenate in sera of two patients suffering from chronic uraemia before and after haemodialysis. After haemodialysis,

| TABLE 1 |
|-----------------|-----------------|-----------------|
| 0.1 ml ‘fraction III’ from | O₂- | P/O- |
| serum equivalent 0.4 ml native serum | consumption | quotient |
| ‘fraction III’ control serum, 0.1 ml | 15.2 | 1.80 |
| ‘fraction III’ control serum, 0.2 ml | 16.4 | 1.95 |
| ‘fraction III’ uraemic serum, 0.1 ml | 10.3 | 0.80 |
| ‘fraction III’ uraemic serum, 0.2 ml | 8.7 | 0.55 |

Respiration and oxidative phosphorylation in kidney mitochondria.
Gas phase O₂, 20 min. incubated, 30°C.
Substrate: 50 µMol DL-β-hydroxybutyrate.
O₂ as µAtom/mg mitochondrial-N, P as µMol/mg mitochondrial-N.
utilization of pyruvate and gluconeogenesis by kidney cortex slices and utilization of glucose and formation of amino acids by brain homogenate was improved.

Fig. 2. Metabolic changes of rat kidney cortex slices (consumption of pyruvate, formation of glucose and lactate) and of brain homogenate (consumption of glucose, consumption and formation of amino acids), before and after haemodialysis. Mean values and S.D.

Attempting to interpret the mentioned findings, one may consider the following points:

1. There is reason to assume that diminished utilization of pyruvate, \(\Delta^+\)-\(\beta\)-hydroxybutyrate and acetoacetate is accompanied by a failure in formation of acetyl coenzyme A, their common metabolic product.

2. Uncoupling of respiration causes a decrease of ATP synthesis and, consequently, a deterioration in the energy supply of the cell.

3. As gluconeogenesis depends upon ATP as energy source, its decrease may be in part explained as due to the reduction in ATP synthesis. In part it may be interpreted, as due to an inhibition of pyruvate carboxylase, an enzyme recently isolated by Utter and Keech (1963). Pyruvate carboxylase is required in synthesis of oxaloacetate. Its cofactor is acetyl coenzyme A, the formation of which is probably decreased in uraemia, as has been pointed out.

4. The amelioration of the observed disturbances which is seen after haemodialysis suggests the presence of low molecular dialysable compounds with toxic effects on cell metabolism. The isolation of a fraction containing most of the toxic action further supports this assumption.
REFERENCES


DISCUSSION

The Chairman: Would anyone like to ask questions about the line of approach that Dr. Renner has been telling us about?

Dr. E. Kemp (Copenhagen): Would you please tell us about your results in acute tubular necrosis?

A second question—what molecular weight had this toxic substance, because you did this sephadex chromatography and I think that you can tell us?

Dr. D. Renner (Frankfurt/Main): The first question: The results in patients suffering from acute anuria, due to tubular necrosis, do not differ from the results in the sera of normal healthy persons.

The second question: I do not know the molecular weight. We have not identified this substance sufficiently. Investigations into this problem have started.

Dr. J. S. Cameron (London): I wanted to ask if you had measured lactate production by these slices or the homogenates.

Dr. D. Renner (Frankfurt/Main): The lactate production is, in the case of sera from patients suffering from chronic uraemia, usually increased. There is, in most cases, an increase of lactate production, using pyruvate as the substrate.

Dr. J. S. Cameron (London): Your results are very interesting but very difficult to interpret. Why I asked about the lactate was because one would expect, if there were a block at the level of pyruvate, or in the utilisation of pyruvate, firstly that you might get lactate accumulating, as you found, but secondly, you might also expect, in the absence of much ATP from the Krebs cycle, an increase in the activity of the Embden-Meyerhof pathway which would result in an increased glucose consumption. Yet I believe you found a decreased glucose consumption.

Dr. D. Renner (Frankfurt/Main): The decrease in formation of glucose you heard...

Dr. J. S. Cameron (London): A decrease in the formation of glucose, not in glucose consumption, I see.

Dr. D. Renner (Frankfurt/Main): Yes, we found a decrease in formation. For formation of glucose, ATP is required and ATP synthesis in uraemia is probably lowered, based on our results. Failure in ATP synthesis causes a decrease in synthesis of glucose. This is the first part of our explanation.

The second part is probably, or possibly, an inhibition of pyruvate carboxylase, which was isolated recently, for pyruvate carboxylase needs as co-factor acetyl co-enzyme A, and acetyl co-enzyme A synthesis is lowered, too, in our experiments.

I do not know if a lowered ATP synthesis or inhibition of pyruvate carboxylase is the only cause—probably both.

Dr. J. S. Cameron (London): Thank you, that answers my question.