Middle Molecules and Haemoglobin Synthesis

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Summary

A modified method for isolating middle molecular substances from uremic
fluids and normal urine using ultrafiltration and Sephadex G-15 Gelfiltration
with a double column system is described.

Using this separation method two peptide-containing fractions with a molecular
weight between 1000 and 1400 daltons were isolated from uremic serum,
dialysate and urine. These fractions were not present in normal serum. Thin-
layer chromatography revealed that most of the isolated fractions consisted of
several components. The middle molecular fractions from uremic fluids and
urine were shown to influence two important steps towards haemoglobin
synthesis (porphobilinogen and globin synthesis), when added to the assay
systems in vitro, but they altered neither porphyrin synthesis nor ATPase
activity in red blood cells. Our results support the assumption that middle
molecular solutes may be important as uremic toxins, especially with regard
to uremic anaemia.

Introduction

The existence of middle molecular substances in uremic blood fluids is
reported in several papers (Dall'Aglio et al, 1972; Dzürık et al, 1973; Fürst
et al, 1975; Man et al, 1973; Migone et al, 1975). In two previous papers (Leber
et al, 1974; Leber et al, 1975) we demonstrated that two important steps
necessary for adequate haemoglobin synthesis are disturbed in the uremic
state: 1. The activity of two enzymes involved in porphyrin synthesis, the
δ-aminolaevulinic acid dehydrogenese and the porphobilinogen desaminase were

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significantly lower in reticulocytes from uraemic patients compared with those from healthy subjects. 2. We proved that globin synthesis is altered in uraemia.

The present investigations were performed to clarify whether middle molecular solutes, isolated by a modified separation method, can influence the activity of these two enzymes involved in porphyrin synthesis in a similar way to that we have found in uraemia.

In addition, the influence of middle molecules on the globin synthesising activity of peripheral blood cells was investigated.

Finally, we measured the activity of ATPase in erythrocytes with and without adding middle molecules to our in vitro assay system.

METHODS AND RESULTS

Figure 1 illustrates our separating system. We used Sephadex G-15 chromatography with a double column and absorption was measured simultaneously at 206 and 254 nm, using a five times scale expansion. In contrast to Fürst (1975) a longer separation time was necessary, but our primary separation shows 20 peaks, eight of them in the middle molecular range.

Figure 1. Sephadex G 15-separating system for 'middle molecules'.

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The middle molecular range was marked in our studies by using Angiotensin I, II, III, Bacitracin, Vasopressin and Tetrogastrin as reference substances. Figure 2 demonstrates all the peaks detected after Sephadex Gelfiltration of uraemic serum, normal serum and normal urine. Peaks two to ten include middle molecular substances. This finding is demonstrated in an original chromatogram

![Chromatogram](image)

Figure 2. Chromatograms (E, 206 nm) of eluates from uraemic serum, normal serum and normal urine (Sephadex G 15, double column system).

![Chromatogram](image)

Figure 3. Original chromatograms of the 'middle molecular' range (E, 206 nm) from normal and uraemic serum.
of the middle molecular range (Figure 3). Uraemic serum contains two peaks which are identified as six and eight. They are not detectable in serum from subjects with normal kidney function. Furthermore the amplitudes of the diverse middle molecular peaks of uraemic serum are significantly higher than those from the serum of healthy subjects. Thin-layer chromatography of freeze-dried eluates indicates that most of the peaks detected in the middle molecular range consist of several ninhydrine positive substances (Figure 4). The shaded spots were found in the eluates of uraemic fluids only.

In our further experiments we found that none of the middle molecular fractions isolated from normal serum had any influence on the enzyme $\delta$-aminolaevulinic acid dehydrogenase. But there was a distinct depression of enzyme activity after the addition of middle molecules from uraemic serum (Figure 5). Peaks five, six and eight exerted the most pronounced inhibitory effect.

The activity of $\delta$-aminolaevulinic acid dehydrogenase was not influenced when low molecular weight solutes were added to the in vitro assay system. Substances known to be retained in renal failure such as guanidines, urea, creatinine, phenolic acid, aromatic or aliphatic amines were investigated. Globin synthesising activity was measured by the $^{14}$C-Histidine uptake. Our results demonstrate a marked inhibition of the $^{14}$C-Histidine uptake when middle molecular fractions from uraemic serum were added to the assay system in vitro.

Globin synthesis decreased in the presence of methylguanidine or guanidino-
Figure 5. Influence of different fractions from gelfiltrated uraemic and normal serum on the activity of δ-aminolaevulinic acid dehydrogenase in vitro.
succinic acid as well. No alteration of globin synthesising activity was found after
the addition of Sephadex filtrates from normal serum or of creatinine or uric
acid. Urea caused a slight but significant increase of globin synthesis. In further
experiments we found no influence of uraemic middle and small molecules on
the activity of the enzyme porphobilinogen desaminase and erythrocytic ATPase.

In conclusion, this is the first time that middle molecular substances from
uraemic fluids could be demonstrated to influence two important steps towards
the synthesis of haemoglobin (porphobilinogen synthesis and globin synthesis).
We are aware that one has to be careful when transferring results from in vitro
experiments to in vivo conditions, but our results support the hypothesis that
uraemic middle molecules may be toxic, and contribute to uraemic anaemia.

References

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Open Discussion

HARTITZSCH (Tulsa) I note your caution in applying in vitro results to the
clinical situation. If middle molecule depression of haemoglobin synthesis is
important in the clinical situation, it must occur in association with depression
of red cell protein synthesis, otherwise we would have a hypochromic anaemia
rather than a normochromic normocytic anaemia in renal failure.

GOUBEAUD Yes, I think it is only one aspect. We have only measured the
uptake of one or two amino acids.