EFFECT OF MEPROMABATE THERAPY ON THE ESTIMATION OF 17-KETOSTEROIDS AND 17-KETOGENIC STEROIDS

By Sigbjörn Salvesen and Roar Nissen-Meyer

In urines obtained from patients treated with meprobamate unexpected low or negative values of 17-ketosteroids (17-KS) and 17-ketogenic steroids (17-KGS) have been found in our laboratories, especially when the correction formula of Gibson & Evans (1937) was used in the final colorimetric assay.

Meprobamate (2-methyl-2n-propyl-1,3-propanediol di-carbamate) is one of the new «tranquillising drugs», and is now widely used in mental hospitals, in medical and surgical departments, and in ambulant practice.

The usual Zimmermann reaction was tried on a pure preparation of meprobamate, and a characteristic colour with an absorption maximum at 395 mµ was obtained. The optical density at 420 mµ was also relatively high, but between 490 and 550 mµ the absorption curve differed only slightly from a straight line. Fig. 1a shows some absorption curves obtained with the Zimmermann reaction performed on different quantities of meprobamate, on dehydroepiandrosterone (DHEA), and on a mixture of these two substances. In Fig. 1b different quantities of meprobamate are plotted against their corrected optical density at 395 mµ with the Zimmermann reaction \(2 \cdot E_{395} - (E_{410} + E_{450}) = E_{\text{corr.}}\). The reaction approximatively follows Beer's law, and may be used in the quantitative estimation of meprobamate.

The Zimmermann reaction on urine extracts from patients treated with meprobamate always gave absorption curves very similar to those given by a mixture of DHEA and meprobamate (Fig. 1a, curve 5).

In Fig. 2 are shown characteristic absorption curves from the same subject before and after taking a usual therapeutic dose of meprobamate. It seems to be obvious that meprobamate is excreted in the urine, and is found in the final extracts for colorimetric assay of 17-KS according to the method of Vestergaard.
Fig. 1 a and b.

a: Absorption curves with the Zimmermann colour reaction.
   I: Meprobamate 0.250 mg.
   II: Meprobamate 0.500 mg.
   III: Meprobamate 0.750 mg.
   IV: Meprobamate 1.000 mg.
   V: Meprobamate 1.000 mg. + DHEA 0.100 mg.
   IV: DHEA 0.100 mg.

b: Calibration curve for meprobamate (pure substance).
Absorption curves with the Zimmermann colour reaction.

I: DHEA 0.033 mg.
II: Male, age 37, volume of urine 1400 ml./24 hrs.
III: Male, age 37, volume of urine 2100 ml./24 hrs., on meprobamate 400 mg. X 5.

(1951), in the assay for 17-KGS according to a modification of the method of Norymberski described by Diezfalusy et al. (1955), and in the assay for 17-hydroxycorticosteroids (17-OHCS) according to the method of Appleby et al. (1955).

Meprobamate appears in the urine as early as the first day of administration, but already on the second day after cessation of treatment no significant amounts appear to be left in the urine (Figs. 3 and 4, Table 1).
Absorption curves with the Zimmermann colour reaction on the 17-KS fractions (Fig. 3) and on the «Total 17-KS fractions» (Fig. 4) from a male, age 37.

I: 1st day, volume of urine 1850 ml./24 hrs., no medication.
II: 2nd day, volume of urine 1930 ml./24 hrs., meprobamate 400 mg. × 5.
III: 3rd day, volume of urine 1730 ml./24 hrs., meprobamate 400 mg. × 5.
IV: 4th day, volume of urine 1920 ml./24 hrs., meprobamate 400 mg. × 5.
V: 5th day, volume of urine 1870 ml./24 hrs., no medication.
VI: 6th day, volume of urine 2400 ml./24 hrs., no medication.

The high optical density of meprobamate at 420 mµ in the Zimmermann reaction makes the use of Gibson and Evans’ correction formula (readings at 420 and 520 mµ) clearly valueless in urine from meprobamate-treated patients. The shape of the absorption curves suggests, however, that the application of the correction formula of Allen (1950) might be more useful.

From Fig. 1 a and 1 b it can be seen that the corrected optical density at
Table 1.
Optical densities of the Zimmermann colour reaction on urine extracts from a male, aged 37 (complete absorption curves in Figs. 3 and 4).

<table>
<thead>
<tr>
<th>Day of experiment</th>
<th>Meprobamate (gm.)</th>
<th>Vol. of urine</th>
<th>E_{420}</th>
<th>E_{470}</th>
<th>E_{490}</th>
<th>E_{520}</th>
<th>E_{550}</th>
<th>E_{570}</th>
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<td>1850</td>
<td>.089</td>
<td>.113</td>
<td>.138</td>
<td>.159</td>
<td>.141</td>
<td>.121</td>
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<td>2</td>
<td>2</td>
<td>1930</td>
<td>.231</td>
<td>.137</td>
<td>.141</td>
<td>.146</td>
<td>.125</td>
<td>.105</td>
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<tr>
<td>3</td>
<td>2</td>
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<td>.345</td>
<td>.174</td>
<td>.167</td>
<td>.168</td>
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<tr>
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<td>2</td>
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<td>.316</td>
<td>.160</td>
<td>.155</td>
<td>.157</td>
<td>.132</td>
<td>.111</td>
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<tr>
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<td>0</td>
<td>1870</td>
<td>.201</td>
<td>.148</td>
<td>.161</td>
<td>.172</td>
<td>.149</td>
<td>.128</td>
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<tr>
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<td>2400</td>
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<td>.128</td>
<td>.146</td>
<td>.159</td>
<td>.139</td>
<td>.120</td>
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Total 17-KS fraction:

<table>
<thead>
<tr>
<th>Day of experiment</th>
<th>Meprobamate (gm.)</th>
<th>Vol. of urine</th>
<th>E_{420}</th>
<th>E_{470}</th>
<th>E_{490}</th>
<th>E_{520}</th>
<th>E_{550}</th>
<th>E_{570}</th>
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<tbody>
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<td>.074</td>
<td>.062</td>
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<tr>
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<td>2</td>
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<td>.135</td>
<td>.084</td>
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<td>.080</td>
<td>.064</td>
<td>.052</td>
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<tr>
<td>3</td>
<td>2</td>
<td>1730</td>
<td>.177</td>
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<td>.074</td>
<td>.060</td>
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<tr>
<td>4</td>
<td>2</td>
<td>1920</td>
<td>.155</td>
<td>.088</td>
<td>.081</td>
<td>.076</td>
<td>.061</td>
<td>.050</td>
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<tr>
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<td>.094</td>
<td>.099</td>
<td>.100</td>
<td>.085</td>
<td>.072</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>2400</td>
<td>.085</td>
<td>.076</td>
<td>.081</td>
<td>.081</td>
<td>.070</td>
<td>.059</td>
</tr>
</tbody>
</table>

DHEA standard 0.033 mg.:

|                  | .051 | .098 | .126 | .149 | .129 | .109 |

395 μg gives a rough estimate of the concentration of meprobamate in the urinary extracts. Estimated in this way it is found that the final 17-KS fraction on the 3rd and 4th day of the experiment shown in Fig. 3 (on meprobamate 2 gm. daily) contained about 800 μg. meprobamate per cuvette (obtained from 5 ml. urine).

Fig. 1 a shows furthermore that up to 1000 μg. meprobamate per cuvette gives an absorption curve in the Zimmermann reaction slightly concave upwards between 470 and 570 μm. Using the Allen correction formula this deviation from the straight line gives a false low corrected optical density at 520 μm. From our data it can now be calculated that in this case, the Allen correction formula with readings at 470, 520 and 570 μm will give an estimate of the 17-KS excretion/24 hours which is about 5–6 mg. too low. With readings at 490, 520 and 550 μm this error will be about 3–4 mg./24 hours. With the correction formula of Gibson and Evans (readings at 420 and 520 μm) the error in this case would be about 12 mg./24 hours, also with false low values.

Estimated in the same way it is found that the final »Total 17-ketosteroid fractions» from the 3rd and 4th day of the experiment contained about 375 μg. meprobamate per cuvette (obtained from 1.25 ml. of urine). The error (false low values) for this fraction will be as follows: With Allen correction and
readings at 470, 520 and 570 mµ about 11 mg./24 hours, with readings at 490, 520 and 550 mµ about 7-8 mg./24 hours, and with Gibson-Evans' correction (readings at 420 and 520 mµ) about 23 mg./24 hours.

When the 17-KGS are calculated (the difference between the »Total 17-ketosteroid fraction« and the 17-KS) the error will of course not be so great.

The values from the experiment calculated with the different correction formulae are illustrated in Fig. 5. If these values are again corrected according to the above calculated errors, it is found that the excretion of 17-KS and 17-KGS in this experiment were not diminished during the intake of 2 gm. meprobamate per day.

Fig. 5.
Values of 17-KS and »Total 17-KS« from the experiment shown in Figs. 3 and 4 and Table 1.

I: calculated with correction formula of Gibson & Evans, $E_{corr} = 2E_{520} - E_{420}$
II: calculated with the correction formula of Allen, $E_{corr} = 2E_{520} - (E_{470} + E_{570})$.
III: calculated with the correction formula of Allen, $E_{corr} = 2E_{520} - (E_{490} + E_{550})$.
×: Values corrected according to the calculated errors of the correction formulae (cfr. text).
SUMMARY

Meprobamate is excreted in the urine and is found in the final extracts for colorimetry of 17-KS and 17-KGS, giving a characteristic absorption curve with the Zimmermann colour reaction. The effect of meprobamate treatment on the values of 17-KS and 17-KGS obtained with the different correction formulae is discussed.

REFERENCES

Vestergaard, P.: Acta endocrinol. 8, 193, 1951.