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# THE PROGNOSTIC VALUE OF THE HYDROXYAMYLOBARBITONE/ AMYLOBARBITONE RATIO

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An accurate, precise and rapid method for the determination of hydroxyamylobarbitone in urine and plasma has been reported previously.

Further studies on the excretion of amylobarbitone and hydroxyamylobarbitone in cases of therapeutic ingestion, overdose and death from amylobarbitone have led to the introduction of a new concept, the urinary ratio of the concentration of hydroxyamylobarbitone/amylobarbitone.

Its significance when associated with measurable blood amylobarbitone levels has led to the suggestion that this ratio may be more indicative of the patient's condition than that of a blood barbiturate level viewed in isolation. Ratios for patients ingesting 200 mg of sodium amylobarbitone were in the range 0–43.8 without measurable blood levels. Overdoses fell within the range 8.8–92.5 with measurable blood levels, the lower ratios being associated with the deepest coma. Very low ratios between 0 and 15.4 were found from cases of death, again with detectable barbiturate in the blood.

The evaluation of the progress of a patient with a barbiturate overdose is often made on the basis of measured blood levels, as is the efficacy of such regimens as haemodialysis, peritoneal dialysis and forced diuresis. Some measurements of the metabolites and unchanged drug have also been made, but these are by no means common and it seemed to us that one of the major pathways by which the body de-toxifies the barbiturate was worthy of closer investigation.

This communication concerns our findings with amylobarbitone and its easily obtainable metabolite, hydroxyamylobarbitone. It was the logical barbiturate for our initial investigations, since a specific and sensitive gas chromatographic method for hydroxyamylobarbitone had already been reported by *Kamm & Van Loon* (1).

Their method, involving the formation of the trimethylsilyl derivative of the metabolite and subsequent gas chromatography on an SE 30 column, proved unsatisfactory in our hands. We have, therefore, develop-

ed and recently described the gas-liquid chromatography of the unchanged metabolite in plasma and urine on an FFAP column (2). It is capable of measuring 2 $\mu$g in an extract.

Barbiturates are normally extracted from biological material at an acid pH by shaking with an organic solvent followed by back-extraction into dilute sodium hydroxide. Since hydroxyamylobarbitone is hydrophilic in nature, back extraction gave low recoveries so that this common purification step could not be used. It was found necessary also, to devise two different procedures for plasma and urine in order to obtain good chromatograms. The steps necessary to obtain these clean extracts are shown in Figure 1.

For plasma, the proteins are precipitated with tungstic acid and the supernatant saturated with ammonium sulphate before extracting with ether and subsequent gas chromatography.

Urine was purified by passing it through a florisil column; an aliquot was then made alkaline with dilute sodium hydroxide, saturated with ammonium sulphate and extracted twice with ether; the pH of the extraction is 8.9. The exact details of these two procedures have been reported by *Grove & Toseland* (2).

Figure 2 shows the chromatogram obtained from an extract from 2 ml of urine from a volunteer who had taken 200 mg of sodium amylobarbitone 24 hours previously.

The fact that barbiturates can be extracted at alkaline pH is not completely new. *Scopp* (3) has measured pentobarbitone at a pH of 9.2. The urine extraction at pH 8.9 gave recovery of 98% for the metabolite, although the recovery for the unchanged drug was only 80%. Hydroxyamylobarbitone was recovered from plasma with an efficiency of 76%.

Table 1 gives the analitical figures obtained in the urine of volunteers receiving 200 mg of sodium amylobarbitone. Hydroxyamylobarbitone was measured up to 6 days after ingestion.

The second column indicates that amylobarbitone can be found up to three days following ingestion. The third column shows the ratio of the concentrations of hydroxyamylobarbitone to amylobarbitone. In the first two days this ratio is usually greater than 15, and in the first three days greater than 10. Maximum blood barbiturate levels were about 0.2 mg% after 1 hour, while that of the metabolite was never greater than 0.5 $\mu$g/ml.

Table 2 shows the ratios obtained from patients taking overdoses of amylobarbitone or Tuinal (a mixture of equal proportions of amylobarbitone and quinalbarbitone). While it may not be strictly true to make direct comparisons between these cases, if one accepts that the hydroxyamylobarbitone/amylobarbitone ratio is a measure of liver function, then a striking pattern emerges.

The numerical value of the ratio, viewed in isolation is as meaningless as a single blood barbiturate level. However, the combination of the ratio and *a measurable blood barbiturate* level is indicative of the condition of the patient.

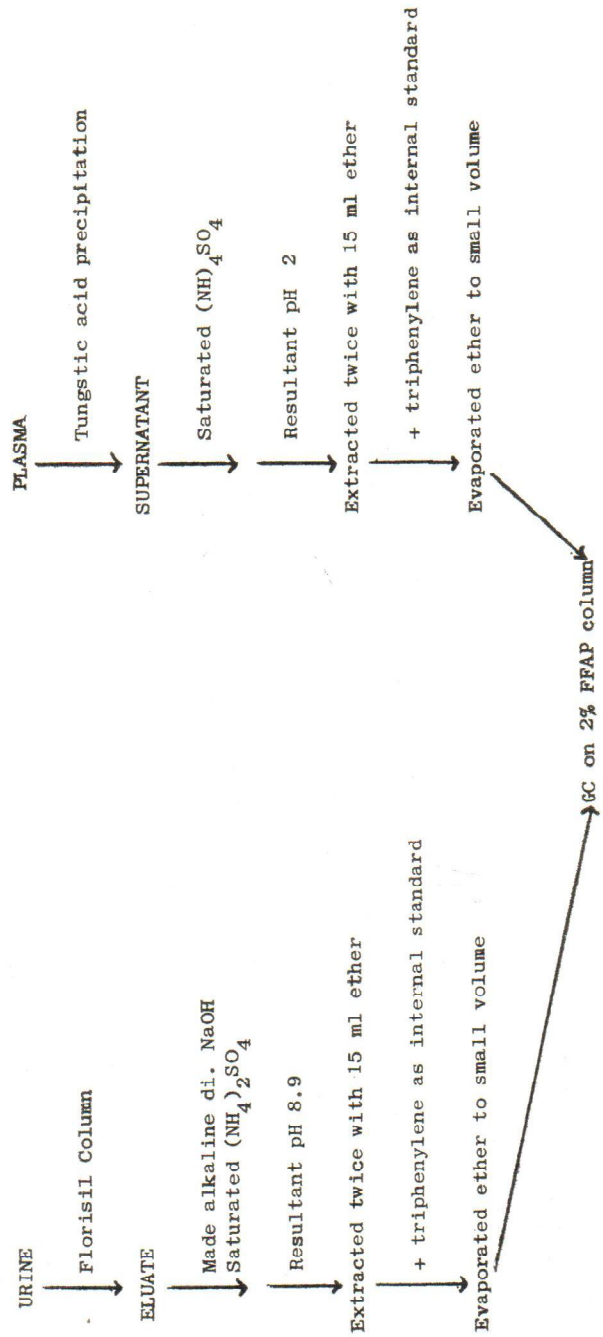


Fig. 1. *Extraction procedures for Hydroxyamylobarbitone from biological material*

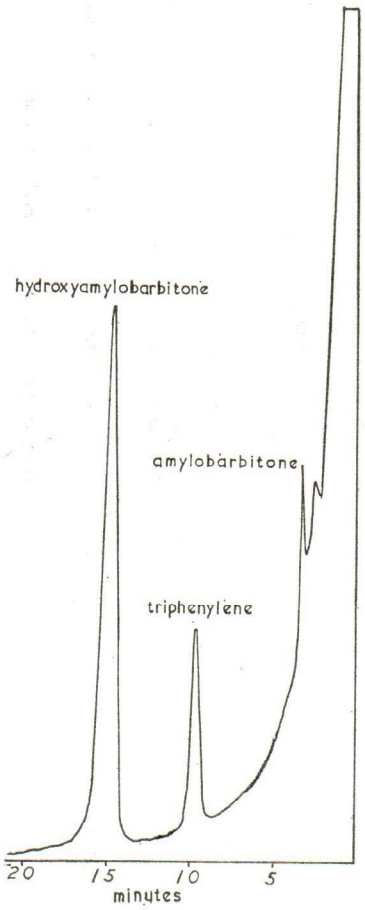


Fig. 2. *A chromatogram obtained from 2 ml of a volunteer's urine, who had taken 200 mg sodium amylobarbitone 24 hours previously*

Hence low ratios associated with measurable blood barbiturate levels are a feature of the patient admitted in coma; the lower the ratio the deeper the coma. Then, as the patient begins to recover more barbiturate is eliminated by hydroxylation and the ratio begins to increase, figures in the order of 80–90 being the maximum found in the cases examined so far. Blood barbiturate levels have not been included in this table in order to simplify its presentation. The figures in italics indicate the periods where measurable levels were obtained, i. e. greater than 0.5 mg%. Patients 3 and 5 were admitted with levels of 1.2 and 1.5 mg% respectively. They have low ratios initially but quickly recover cons-

Table 1

*Excretion of Amylobarbitone and Hydroxyamylobarbitone in volunteers' urine after ingestion of 200 mg of sodium amytal*

| | % Dose as Hydroxyamylobarbitone on day: | | | | | | | % Dose as Amylobarbitone on day: | | | | | | | Ratio HO-A/A on day: | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | Total | 1 | 2 | 3 | 4 | 5 | 6 | Total | 1 | 2 | 3 | 4 | 5 | 6 | Total |
| V1 | 12.32 | 11.87 | 5.73 | 2.66 | 1.40 | 0 | 33.98 | 0.45 | 0.38 | 0.15 | 0.13 | 0 | 0 | 1.11 | 27.4 | 31.2 | 38.2 | 20.5 | | | 30.6 |
| V2 | 9.68 | 9.26 | 6.63 | 3.97 | 3.17 | 2.46 | 35.17 | 1.11 | 0.55 | 0.63 | 0.34 | 0 | 0 | 2.63 | 8.7 | 17.5 | 10.5 | 11.7 | | | 13.4 |
| V3 | 18.36 | 19.21 | 5.88 | 1.51 | 2.59 | 0.95 | 48.50 | 0.90 | 0.44 | 0.60 | 0.70 | 0.48 | 0 | 3.12 | 20.4 | 43.8 | 9.8 | 2.2 | 5.4 | | 15.5 |
| V4 | 13.06 | 13.69 | 9.41 | 4.15 | 2.42 | 2.33 | 45.06 | 1.32 | 0.84 | 0.44 | 0.25 | 0.13 | 0.09 | 3.07 | 9.9 | 16.3 | 16.8 | 16.6 | 18.6 | 25.9 | 14.7 |
| V5 | 10.26 | 13.43 | 10.01 | 4.67 | 1.14 | 0.67 | 40.18 | 0.30 | 0.60 | 0.25 | 0 | 0 | 0 | 1.15 | 33.4 | 22.4 | 40.0 | | | | 34.9 |

Table 2

*Ratios of Hydroxyamylobarbitone/Amylobarbitone in urine from patients taking overdoses of Amytal or Tuinal*

| Patient | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 |
|---|---|---|---|---|---|---|---|---|
| 1. Amylobarbitone | *44.8* | *73.4* | *41.0* | 70 | 81 | 7 | 16 | 7.5 |
| 2. Tuinal | *18.75* | *58.2* | *10* | *8.8* | 2.5 | | | Died |
| 3. Amylobarbitone | 0– 8 hr *12.7*<br>8–16 hr 80.2<br>16–24 hr 56.4 | | | | | | | |
| 4. Amylobarbitone | *14.5* | *15.9* | *34.4* | *92.5* | 80.9 | 65.7 | | |
| 5. Amylobarbitone | 8–16 hr *38.4*<br>16–24 hr 37.9 | 0– 8 hr 24.3<br>8–16 hr *24.3*<br>16–24 hr 37.9 | | | | | | |

Figures in italics indicate blood levels over 0.5 mg %

Table 3

*Some cases of Amytal and Tuinal deaths*

| Case | Age & Sex | Barbiturate Blood Level | Hydroxyamylo-barbitone Blood level | Amylobarbitone Urine level | Hydroxyamylo-barbitone Urine level | Urine Ratio HO-A/A |
|---|---|---|---|---|---|---|
| 1 | 30 M | 2.1 mg% Amylobarbitone | — | 2.92 mg% | 12.8 mg% | 0.44 |
| 2 | 60 F | 3.0 mg% Amylobarbitone | 0.22 mg% | 1.16 mg% | 1.67 mg% | 1.4 |
| 3 | 36 M | 2.4 mg% Amylobarbitone<br>1.8 mg% Quinalbarbitone | 0.29 mg% | 0.34 mg% | 1.2 mg% | 3.5 |
| 4 | 29 F | 5.1 mg% Amylobarbitone | 1.65 mg% | 0.87 mg% | 13.4 mg% | 15.4 |
| 5 | 21 F | 2.5 mg% Amylobarbitone<br>1.4 mg% Quinalbarbitone | 1.10 mg% | 1.69 mg% | 5.56 mg% | 3.3 |
| 6 | 49 F | 16.6 mg% Amylobarbitone | None detected | 2.8 mg% | None detected | 0 |

ciousness with the ratio rising to a maximum of 80.2 and 87.3 respectively. Patients 1, 2 and 4 show the same kind of pattern over a longer period. The patients were admitted with levels of 3, 3.5 and 4 mg% respectively. Patient 1 was, even on admission, dealing with the drug perfectly satisfactorily, the high ratios giving no cause for alarm. Patient 2, on the other hand, appeared to be recovering on day 2, suffered a cardiac arrest, and as these extremely low ratios show, further metabolism was reduced to a low level until she died on day 8. Patient 4 actually had an increase in blood barbiturate level on day 2, and this is reflected in the low ratios, until again consciousness was recovered and a large increase in metabolism is observed on day 4.

The pattern of low ratios and measurable blood levels is indicative of the body not being able to detoxify the drug adequately. Table 3 shows results of analyses of blood and urine in six deaths from overdose. In all cases, the hydroxyamylobarbitone/amylobarbitone ratio is low, in fact in case 6 metabolism has not even started. Hydroxyamylobarbitone was not found in either blood or urine. The massive overdose presumably inhibited hydroxylation completely.

Figure 3 is a simplified diagram summarising the value of this ratio when associated with measurable blood levels. The change in this ratio, together with the presence of unmetabolised drug is indicative of the way in which the patient is detoxifying the amylobarbitone. If the patient is successfully metabolising the drug, the ratio will rise to a maximum of 80–90 as consciousness approaches and then decrease over 5–6 days. since there is little or no barbiturate in the body to metabolise. On the other hand, patients in deep coma may have ratios in the order of 15–30 for several days before recovering consciousness. Patients who fail to metabolise the drug have large amounts of unchanged barbiturate in their urine and hence low ratios are associated with cases of death.

*Jackson & Moss* (4) identified hydroxyamylobarbitone in the urine of a woman who had died 30 hours after ingesting Tuinal. They did not find any barbiturate in the blood, viscera or urine. This is surprising, and that death may be justifiably attributed to barbiturate poisoning without demonstrating the presence of unchanged barbiturate, is unwise in our opinion.

Amylobarbitone has been shown by *Cochin & Daly* (5) and *Kamm* and *Loon* (1) to be present in the early morning urine of patients receiving therapeutic doses. Table 1 shows also that amylobarbitone can be found to be present up to three days later. The presence of hydroxyamylobarbitone without that of amylobarbitone must surely indicate that the body has detoxified any barbiturate and the cause of death must lie elsewhere.

In conclusion, for the clinician we advocate that a blood amylobarbitone ratio is of more prognostic value than the conventional blood level alone. Persistent low ratios may possibly be considered as an indication for the need for diuresis or dialysis.

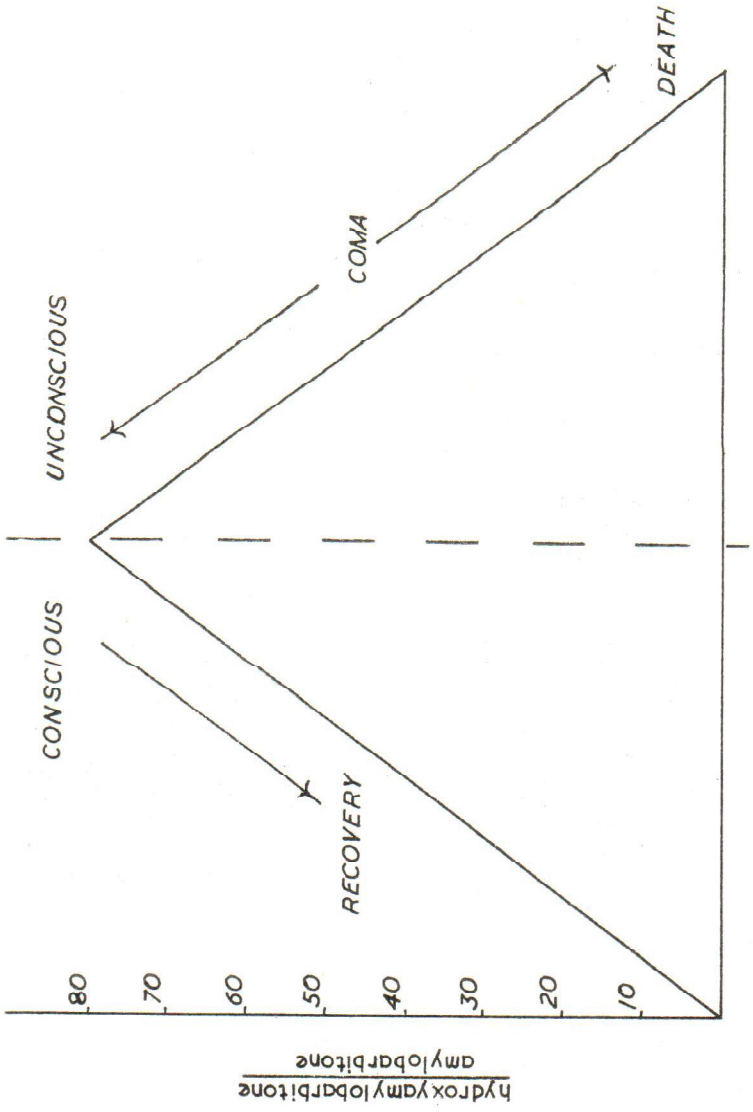


Fig. 3. *A schematic diagram showing the tertiary relationship between the urinary hydroxyamylobarbitone/amylobarbitone ratio, measurable blood levels and the condition of the patient*

The pathologist may also like to examine the value of this ratio. It is probably more conclusive to be able to indicate that the functioning of the liver has been retarded than to attempt to draw inferences from low blood barbiturate levels.

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*References*

1. *Kamm, J. J., Van Loon, E. J.:* Clin. Chim. Acta, *12* (1966) 789.
2. *Grove, J., Toseland, P. A.:* Clin. Chim. Acta, *29* (1970) 253.
3. *Scoppa, P.:* Biochim. appl., *13* (1966) 274.
4. *Jackson, J. V., Moss, M. S.:* Nature, *192* (1961) 553.
5. *Cochin, J., Daly, J. W.:* J. Pharmac., *139* (1963) 154.

*Sažetak*

VRIJEDNOST ODNOSA HIDROKSIAMILOBARBITONA I AMILOBARBITONA U URINU ZA PROGNOZIRANJE ISHODA OTROVANJA

Točna i brza metoda određivanja hidroksiamilobarbitona u urinu i plazmi već je ranije opisana.

Daljim istraživanjima ekskrecije amilobarbitona i hidroksiamilobarbitona u slučajevima terapije amilobarbitonom, te uzimanja prekomjerne doze i fatalnog trovanja amilobarbitonom uveden je novi pojam – izražavanje koncentracije hidroksiamilobarbitona i amilobarbitona u urinu u obliku omjera.

Čini se da bi ovaj omjer povezan s količinom amilobarbitona u krvi mogao biti mnogo bolji pokazatelj pacijentova stanja od količine barbiturata u krvi promatrane odvojeno. U pacijenata koji su uzeli 200 mg natrijevog amilobarbitona omjeri su bili između 0 i 43,8 a količina barbiturata u krvi nije bila uvjerljiva, s tim da su niži omjeri utvrđeni u slučaju najdublje kome. Vrlo niski omjeri između 0 i 15,4 nađeni su u slučajevima smrti, a tada su barbiturati bili mjerljivi u krvi.

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