From our present data we are unable to provide an explanation for these differences but a number of possibilities might be considered. Firstly there is the possibility of competition for lipid storage sites between ether and the cannabinoids, however, from structural considerations the different cannabinoids would be expected to behave similarly. Secondly there is the possibility of an inhibition by the cannabinoids of the metabolism of pentobarbitone, but not of ether which is not metabolized (Paton & Pertwee, 1972) and thirdly, different interactions in the central nervous system between the cannabinoids and the different anaesthetics might occur. Such possibilities deserve further investigation because of their potential clinical and social significance.

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Department of Pharmacology,
University of Sydney,
Sydney, N.S.W., 2006, Australia.

R. MALOR
D. M. JACKSON
G. B. CHESHER

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Effect of *in vitro* changes in urinary pH on the enzymatic measurement and daily variation in excretion of D-glucaric acid

Latham (1974a) suggested that the measurement of urinary D-glucaric acid, by an enzymatic assay adapted from that of Marsh (1963), was a reliable test of hepatic enzyme induction after the administration of anticonvulsant drugs to man. Preceding these studies, several workers (Mowat, 1968; Hunter, Carrella & others, 1971; Hunter, Maxwell & others, 1972; Latham, Millbank & others, 1973) have used this or a similar technique (Marsh, 1963) without establishing limitations of the methodology. Recently, Simmons, Davis & others (1974) suggested that the procedure of Marsh (1963) allowed an increase in urinary pH on boiling in acid. This effect was thought to interfere with the final D-glucaric acid concentration obtained because the original assay relied on the conversion of D-glucaric acid to the β-glucuronidase inhibitor D-glucaro-(1–>4)-lactone, the lactonization being pH dependent. Confirmation of these observations could impair the validity of the method previously described by Latham (1974a), which used identical buffers to those described by Marsh (1963), unless a satisfactory alternative explanation could be established.

The effect of the inherent buffering properties of different urine specimens on the daily variation in the measured excretion of urinary D-glucaric acid in normal

subjects and epileptic patients receiving anticonvulsant drug therapy was therefore determined. Whether the apparent daily variation in urinary D-glucaric acid resulted from the different inherent buffering properties of urines or from some other cause could, therefore, be clarified. The absence of a relation between these effects would suggest that changes in urinary pH during the boiling procedure (Marsh, 1963; Latham, 1974a) do not affect the final D-glucaric acid result.

Six male epileptic patients receiving various combinations of anticonvulsant drug therapy and 6 normal (i.e. drug free) male subjects supplied 24 h urine collections for up to 7 consecutive days. To minimize any interbatch variation, aliquots of the 24 h specimens (of known total volume) from each subject were stored at −15° and assayed simultaneously. Urinary D-glucaric acid concentrations (as D-glucaro-(1−>4)-lactone) were determined in duplicate by the method of Latham (1974a).

Aliquots of spot urine collections, provided by 6 normal male subjects were adjusted to pH 2·0 with HCl and heated in a boiling water bath for up to 40 min. One aliquot from each subject was removed after heating for predetermined intervals of time, cooled for 5 min and the pH recorded at room temperature.

The experiment was repeated using aliquots of 24 h urine collections supplied by 6 epileptic patients who were receiving various combinations of anticonvulsant drug therapy. The specimens were selected on a random basis with respect to drug treatment. The procedure further differed from the previous experiment because all the specimens were aliquots from complete 24 h urine collections and were stored at −15°. The final pH after heating (initially at pH 2·0) for 40 min was recorded for aliquots of 24 h urine collections from an additional 10 epileptic patients and 10 normal subjects, the samples having been stored at −15°.

In another experiment, the final pH of the aliquots of 24 h urine collections (after heating for 40 min starting at pH 2·0), provided for up to 7 consecutive days by epileptic patients and normal subjects, was recorded before determination of the D-glucaric acid concentration.

The mean coefficient of variation in the daily excretion of D-glucaric acid measured in epileptic patients on consecutive days was 40·7% (Table 1). In normal subjects, the mean coefficient of variation in the excretion of D-glucaric acid on 7 consecutive days was 41·1% (Table 2).

Heated urine specimens, initially at pH 2·0, for up to 40 min produced significant increases in pH (spot normal, $t=4{\cdot}44$, $P<0{\cdot}01$; 24 h epileptic, $t=4{\cdot}60$, $P<0{\cdot}001$) between the 10 and 40 min samples (Fig. 1). Furthermore, there were significant

Table 1. *Variation in the daily excretion of* D-*glucaric acid in 6 male epileptic patients receiving anticonvulsant therapy.*

	Epileptic patients					
Day	1	2	3	4	5	6
1	90	193	76	130	166	374
2	153	258	75	291	73	227
3	121	266	117	763	138	128
4	82	174	86	264	69	217
5	5)	—	138	367	161	400
6	72	—	44	—	251	—
7	92	—	—	—	128	—
Mean	95·6	222·8	89·3	363·0	140·9	269·2
s.d.	31·8	46·1	33·4	239·4	62·1	114·6
cv %	32·3	20·7	37·4	66·0	44·1	42·6

Mean coefficient of variation (cv) = 40·7%.

Table 2. *Variation in the daily excretion of* D-*glucaric acid in 6 normal subjects.*

	Normal subjects					
Day	1	2	3	4	5	6
1	1·7	7·4	8·6	5·5	7·3	2·6
2	2·2	5·3	8·5	4·9	19·7	4·3
3	2·7	9·7	5·7	6·3	12·3	3·8
4	5·6	8·7	5·9	10·1	11·6	0·6
5	5·8	9·3	7·7	10·4	3·1	1·8
6	6·6	6·7	7·6	4·6	11·5	4·0
7	5·4	16·5	5·7	6·5	7·0	1·2
Mean	4·29	9·09	7·10	6·90	10·36	2·61
s.d.	2·00	3·62	1·30	2·39	5·28	1·47
cv %	46·8	39·8	18·3	34·6	51·0	56·3

Mean coefficient of variation (cv) = 41·1 %.

increases in variance ratios between the 10 and 40 min samples for both groups (spot normal, $F=22{\cdot}4$, $P<0{\cdot}01$; 24 h epileptic, $F=17{\cdot}40$, $P<0{\cdot}01$). Although there was a trend towards a reduced increase in the final pH of the 24 h epileptic urines after treatment compared with the normal spot specimens the difference between the means or variance ratio was not significant ($t=1{\cdot}97$, $P>0{\cdot}05$; $F=4{\cdot}06$, $P>0{\cdot}05$). The final mean pH after boiling aliquots of 10 normal (24 h) and 10 epileptic (24 h) specimens for 40 min was similar, there being no significant difference between them ($t=0{\cdot}08$, $P>0{\cdot}1$). Again there was a trend for the mean final pH values to be lower

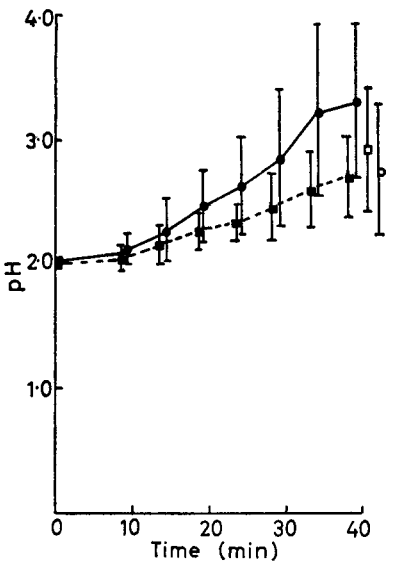

Fig. 1.

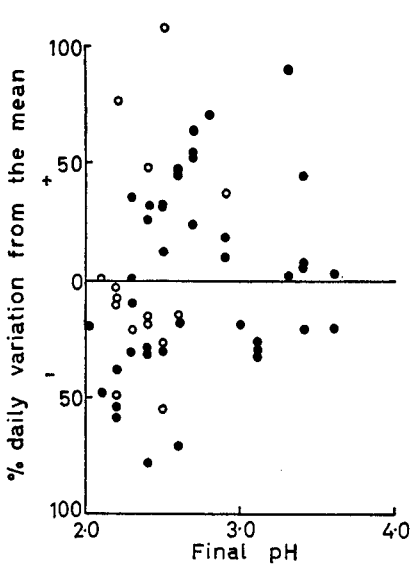

Fig. 2.

FIG. 1. Change in the pH of urine specimens, initially adjusted to pH 2·0 with HCl, after heating for up to 40 min. Spot specimens were provided by 6 normal subjects (●). Aliquots of 24 h urine voids were supplied by an additional 10 normal subjects (○) and groups of 6 (■) and 10 (□) epileptic patients receiving a variety of anticonvulsant drugs. For the 10 normal subjects and 10 epileptic patients, only the final pH (after heating for 40 min) was recorded. Results plotted are the means with s.d.

FIG. 2. % daily change from the mean urinary D-glucaric acid concentration in epileptic patients (○) and normal subjects (●) as a function of the final pH of each specimen after heating for 40 min starting at pH 2·0.

than the mean recorded from spot specimens (Fig. 1), although the differences were not significant ($P>0{\cdot}05$, Student's *t*-test).

The % daily variation from the mean D-glucaric acid concentration for each particular epileptic patient (at least 5 consecutive estimations) or normal subject (7 consecutive estimations) was plotted as a function of the final pH (Fig. 2). There was no obvious correlation between the % change in D-glucaric acid excretion and the final pH of the specimens after boiling for 40 min.

The evidence presented confirms the observation of Simmons & others (1974), there being a marked rise in pH during the boiling procedure after acidifying urine specimens to pH 2·0 with HCl. Although the intrinsic buffering properties of all the specimens varied widely, there was a trend for the 24 h specimens to have a greater inherent buffering capacity. The absence of significantly different final pH or variance ratio values between the urines from epileptics and normal subjects suggested that the anti-convulsant drugs or their metabolites in the urines of epileptic patients did not affect the intrinsic buffering properties of urine. These increases in pH could not be related to the considerable daily fluctuation in the measured excretion of urinary D-glucaric acid. The two effects were, therefore, thought to be independent. Hence the daily fluctuations in urinary D-glucaric acid excretion probably result from genuine *in vivo* biochemical effects rather than artifacts produced by the variable buffering properties of different urine specimens during *in vitro* treatment. The enzymatic methods previously described for the determination of urinary D-glucaric acid (Marsh, 1963; Latham, 1974a) were not, therefore, thought to be adversely affected by the changes in pH which occur during the boiling procedure.

The relative importance of the considerable daily fluctuation in the excretion of urinary D-glucaric acid in both normal subjects and epileptic patients receiving drug therapy would be greater when considering small changes. In these circumstances, lack of significance may be due to this variation rather than the absence of an inducing effect. The assay is, however, sufficiently sensitive to accommodate these anomalies after the chronic administration of anticonvulsant drugs (Latham, 1974b).

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Department of Clinical Pharmacology,
St. Bartholomew's Hospital, London, EC1A 7BE, U.K.

A. N. LATHAM*

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* Present address: Department of Medicine, McMaster University, Hamilton, Ontario, Canada, L8S 4J9.