

biliary L.A. levels have also been described in the rabbit,<sup>4</sup> but have not been observed after the administration of C.D.C.A. in man.<sup>5</sup>

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### PURITY CRITERIA FOR CHENODEOXYCHOLIC ACID

SIR,—In recent reports<sup>6,7</sup> the purity of clinically used chenodeoxycholic acid (C.D.C.A.) was discussed, but a detailed analysis was not given. Presumably in this context Mosbach et al.<sup>8</sup> found it necessary to draw attention to the possibility of the inclusion of crystallisation fluid in C.D.C.A. preparations. This heptane inclusion caused C.D.C.A. to melt at 119°C,<sup>9</sup> which contrasts with the melting-point of 143°C as reported by, for example, Fieser and Rajagopalan.<sup>10</sup> During preparative work on C.D.C.A. we experienced several difficulties and we should like to mention some of the pitfalls to be avoided when assessing the purity of C.D.C.A. preparations.

To prevent some polar products remaining undetected by thin-layer chromatography (T.L.C.), it is in our opinion necessary to dissolve C.D.C.A. batches in methanol and not to subject them to bile-salt extraction. Indeed, polar products could sometimes be found on further analysis by using three different T.L.C. systems, with sufficient resolving power to separate C.D.C.A. from lithocholic, cholic, and deoxycholic acid, and the 3- and/or 7-methyl ether of C.D.C.A. Polar products remain at the start. This prompted us to look for better preparation and crystallisation methods and our searches resulted in C.D.C.A. batches containing less than 0.3% of so-called "side-products" as determined by T.L.C. This was further confirmed by conventional techniques such as high-pressure liquid chromatography of C.D.C.A. as the free acid and by gas-liquid chromatography (G.L.C.) of its methyl ester.

Methyl esterification by using diazomethane gave rise to variable amounts (1–5%) of 3- and 7-methylethers (confirmed by combined G.L.C. mass spectrometry) of C.D.C.A. The presence of these products in the original samples could be excluded with certainty.

Co-crystallised solvent could easily be detected by thermogravimetric analysis since a sharp weight drop occurs at 120°C when solvent is included in the crystals.

By using differential thermal analysis the following transitions were observed with batches of C.D.C.A.: an endothermic process occurs at about 120°C and is the result of release of co-crystallised solvent (see discussion by Mosbach et al.<sup>8</sup>). This is followed by another endothermic reaction at 142–145°C which is in agreement with the melting-point of one crystalline form. Increasing temperature leads to an exothermic reaction between 145° and 160°C corresponding with recrystallisation, which is finally followed by a sharp endothermic transition at 168°C, corresponding with the final melting-point of C.D.C.A. Depending on the purification and crystallisation procedures used, a C.D.C.A.

preparation could be obtained which showed a single and sharp endothermic transition peak at 168°C. From these data it was concluded that C.D.C.A. can exist in at least two crystalline states.

Rigid purity criteria should be used when assessing quality of C.D.C.A. preparations intended for use in man, since some impurities may be potentially toxic. Furthermore, attention should be paid to the crystalline structure, since this may play a role in absorption or in possible gastrointestinal irritation caused by C.D.C.A. preparations.

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### BASE EXCESS AND ORGANIC ACIDÆMIA

SIR,—We read with great interest the article by Dr Howorth.<sup>1</sup> In fact this may be a continuation, or a re-opening, of the so-called transatlantic debate.

Looking into the literature for studies about a correlation between the non-respiratory component of acid-base balance in blood (base excess or buffer base) and the actual change in the concentration of organic acids, the information is very meagre. As far as we know only one study<sup>2</sup> was undertaken where the change of lactic acid in whole blood is correlated with the metabolic change in the bicarbonate concentration of arterial blood. In this study the increase of blood-lactic-acid concentration of the test subjects was provoked by nine minutes of exercise. In this type of in-vivo experiments, where acute changes of acid-base balance occur, these authors found a reasonable correlation (correlation coefficient 0.81) between the two parameters.

We studied several patients, however, with chronic organic acidæmia due to different causes. In fig. 1 the values of base excess and lactic-acid concentration are presented, both determined in whole blood obtained from a patient with chronic lactic acidæmia due to pyruvate-carboxylase deficiency.<sup>3</sup> Arterialised blood was obtained by fingerprick. The lactic-acid concentration was measured in blood according to Hohorst.<sup>4</sup> pH and pCO<sub>2</sub> were measured with the pH-blood-gas analyser (type 313, Instrumentation Laboratories; for pH determination, the reference

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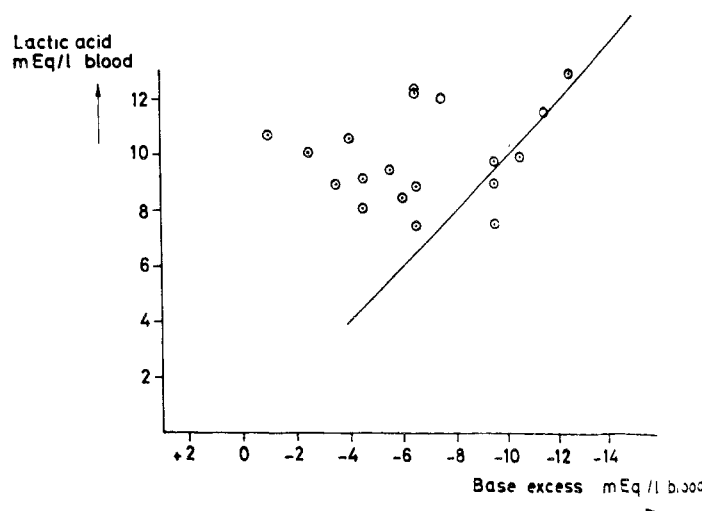


Fig. 1—Relation between base excess and whole-blood lactic acid in a patient with chronic lactic acidæmia due to pyruvate-carboxylase deficiency.

The solid line represents the ideal correlation between base excess and lactic acid.

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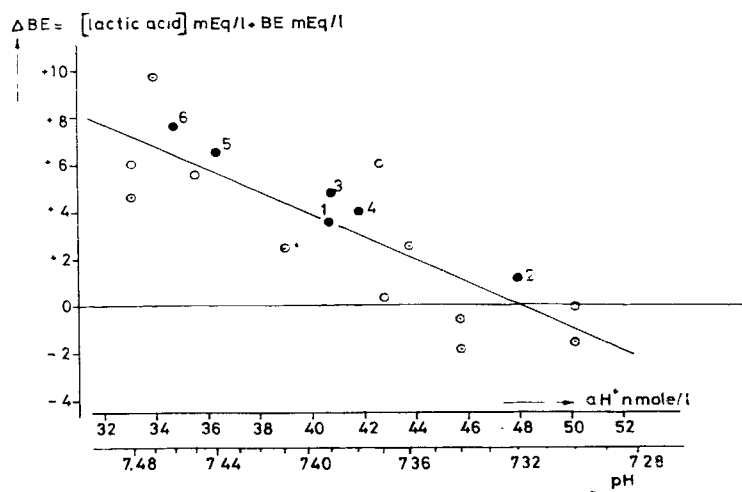


Fig. 2—Relation between hydrogen-ion activity ( $aH^+$  in nmol per litre) and  $\Delta B.E.$  (whole-blood lactic acid and base excess, both in meq. per litre).

These values are obtained from results in fig. 1.

calomel electrode was set at 37°C). The base excess was calculated from the Siggaard-Anderson nomogram.<sup>5</sup> During these observations no neutralising therapy was given. Assuming now that in this case the negative values of base excess must approximately agree with the concentration of lactic acid (solid line in fig. 1), it can be seen that many values deviate from the value to be expected. Moreover, we observed that the more the blood-pH is normalised the more the observed values deviate from the solid line in this figure.

In fig. 2 results of the same patient are demonstrated as ( $aH^+$ ) versus  $\Delta B.E.$ , in which ( $aH^+$ ) represents the hydrogen-ion activity (normal 36–44 nanomole per l.) and  $\Delta B.E.$  the algebraic sum of the lactic-acid concentration and the base excess. Normally, we would expect that  $\Delta B.E.$ , independent of the blood-pH, would be approximately zero. From this figure, however, it can be seen that  $\Delta B.E.$  is not zero under all circumstances and is inversely related with the hydrogen-ion activity. For this relationship between both parameters we calculated the linear algebraic equation:  $\Delta B.E. = -0.48 (aH^+) + 23.2$ , with a standard deviation ( $S_{yx}$ ) of  $\pm 1.75$  and a correlation coefficient of 0.84. The solid circles in this figure represent six observations which are obtained within a period of eight hours, the numbers referring to the order of succession of these observations within this period. The reason for this variation, over a relative short period of time, is obscure to us.

The same phenomena were observed in other cases of organic acidæmia, including methylmalonic acidæmia and lactic acidæmia due to mitochondrial myopathy.<sup>6</sup> Fig. 3 presents the results of a patient suffering from methylmalonic acidæmia. The same discrepancy between base excess and organic acid concentrations was seen. In this figure,  $\Delta B.E.$  represents the algebraic sum of the concentrations of the organic acids in meq. per l. (methylmalonic-, lactic-,  $\beta$ -hydroxybutyric-acid) and the base excess. Again the inverse relationship between  $\Delta B.E.$  and ( $aH^+$ ) is seen. From this data the linear algebraic equation  $\Delta B.E. = -0.65 (aH^+) + 30.7$  (standard deviation ( $S_{yx}$ )  $\pm 1.90$ , correlation coefficient 0.74) was calculated. The organic acids were determined in serum (fasting values, without neutralising therapy) by gas chromatography of their trimethylsilyl derivatives, as described previously.<sup>7</sup>

Serum sodium and chloride concentrations were normal in most instances. No correlation could be detected between  $\Delta B.E.$  and serum-chloride concentration, nor between  $\Delta B.E.$  and the calculated anion gap ( $Na^+ + K^+ - Cl^-$ ).

These observations support the criticism of the base-excess concept, and particularly of its usefulness in the diagnosis of patients with chronic organic acidæmia; and they point to the necessity of gas-chromatographic analysis

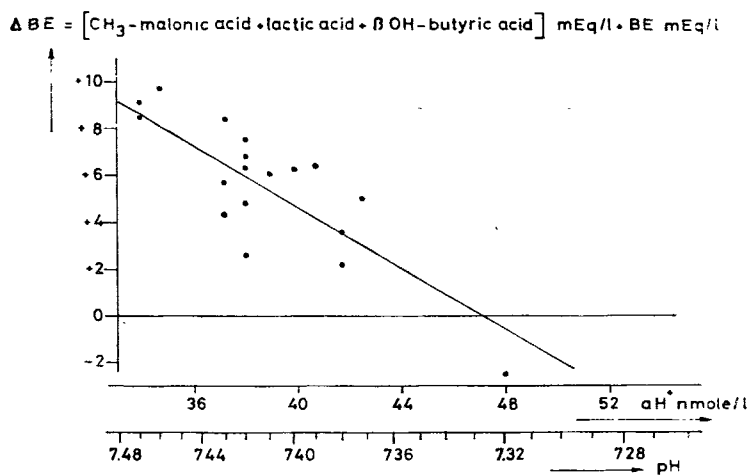


Fig. 3—Relation between the hydrogen-ion activity ( $aH^+$ ) and  $\Delta B.E.$  in a patient with methyl-malonic acidæmia.

In this case  $\Delta B.E.$  is calculated as the algebraic sum of the serum methyl-malonic, lactic, and the  $\beta$ -hydroxybutyric acid concentrations and the base excess, all expressed in meq. per litre.

of blood of patients which are suspected, on clinical grounds, of organic acidæmia.

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## LACTIC ACIDOSIS AND HYPERLACTATÆMIA

SIR,—Dr Kreisberg (Oct. 19, p. 960) has made two further points related to the pathogenesis of lactic acidosis on which we should like to comment. The first is that he doubts whether isolated impairment of hepatic lactate utilisation would result in systemic lactic acidosis. His second point is the suggestion that skeletal muscle would be the tissue which took over the role of lactate utilisation in the event of decreased hepatic lactate uptake.

On theoretical grounds it seems that in these circumstances the most probable stimulus to extrahepatic tissues to increase their lactate utilisation would be a raised blood-lactate concentration, there being no evidence that these tissues can sense the deficiency of lactate utilisation elsewhere by any other means. Thus some degree of lactic acidosis would be inevitable, the amount depending on the ability of extrahepatic tissues to respond to an increased lactate concentration in the blood perfusing them. That compensation for removal of one site of lactate removal is not complete is indicated by the decrease of the rate of removal of a lactic-acid load which is seen in conscious resting nephrectomised rats (compared with sham-operated controls); this slowing is considerably increased in the presence of acidosis.<sup>1,2</sup> Furthermore a decreased rate of removal of a lactate load is seen in patients with liver disease.<sup>3</sup> Dr Kreisberg quotes the work of Tashkin et al.,<sup>4</sup> which shows that the rise of arterial blood-lactate which occurs when the blood-supply to the canine liver is decreased does not reach significance. However, in these studies, the main purpose of which was other than to decide the issue currently under debate, hepatic lactate uptake had only been significantly reduced for approximately 30 minutes at the time when the rise in arterial

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