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Proton-nuclear-magnetic-resonance studies of serum, plasma and urine from fasting normal and diabetic subjects

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Resonances for the ketone bodies 3-D-hydroxybutyrate, acetone and acetoacetate are readily detected in serum, plasma and urine samples from fasting and diabetic subjects by ^{1}H n.m.r. spectroscopy at 400 MHz. Besides the simultaneous observation of metabolites, the major advantage of n.m.r. is that little or no pretreatment of samples is required. N.m.r. determinations of 3-D-hydroxybutyrate, acetoacetate, lactate, valine and alanine were compared with determinations made with conventional assays at six 2-hourly intervals after insulin withdrawal from a diabetic subject. The n.m.r. results closely paralleled those of other assays although, by n.m.r., acetoacetate levels continued to rise rather than reaching a plateau 4h after insulin withdrawal. The 3-D-hydroxybutyrate/acetoacetate ratio in urine during withdrawal gradually increased to the value observed in plasma (3.0 ± 0.2) as determined by n.m.r. The acetoacetate/acetone ratio in urine (17 ± 6) was much higher than in plasma (2.5 ± 0.7). Depletion of a mobile pool of fatty acids in plasma during fasting, as seen by n.m.r., paralleled that seen during insulin withdrawal. These fatty acids were thought to be largely in chylomicrons, acylglycerols and lipoproteins, and were grossly elevated in plasma samples from a non-insulin-dependent diabetic and in cases of known hyperlipidaemia.

We have recently shown that useful biochemical information can be obtained from high-resolution high-field ^{1}H n.m.r. studies of animal serum and plasma (Nicholson *et al.*, 1983). Broad overlapping resonances from high-M_r substances such as albumin and immunoglobulins were effectively suppressed by using the spin-echo Fourier-transform (s.e.f.t.) technique [for reviews, see Rabenstein & Nakashima (1979) and Brown & Campbell (1980)]. This leaves well-resolved peaks in the spectrum from a wide range of low-M_r mobile* metabolites present at millimolar concentrations. Simplification of ^{1}H n.m.r. spectra of biological materials in this way was first reported by Daniels *et al.* (1976). A major attraction of the method is that little or no pretreatment of the samples is required. We have now investigated whether such proton n.m.r. spectra can provide useful 'fingerprints' of the biochemical changes which accompany diseased states (Bock, 1982; Iles *et al.*, 1983).

As an initial study we chose diabetes mellitus, since glucose would be elevated and the ketone bodies 3-D-hydroxybutyrate, acetone and acetoacetate would be expected to be present in plasma at millimolar levels. In addition, ketone bodies all have methyl groups and therefore possess favourable n.m.r. detection characteristics. Comparable levels of ketone bodies are found in plasmas of fasting subjects, but glucose levels are lowered. It was of interest to compare the concentrations of the metabolites as seen by n.m.r. with those determined by conventional chemical assays. For this,

Abbreviations used: s.e.f.t., spin-echo Fourier-transform; n.O.e., nuclear Overhauser effect.

* In this paper the word 'mobile' is used in the n.m.r. sense to describe molecules which undergo rapid molecular motion, e.g. tumbling. Slow motion leads to short T_2 (spin–spin relaxation time) values and broad n.m.r. resonances [for a discussion, see Jardetsky & Roberts (1982)].

samples from an insulin-dependent diabetic subject were monitored over a 13h period at regular intervals after insulin withdrawal. We also show that normal (single-pulse) high-resolution ^1H n.m.r. studies of untreated urine contain a wealth of complementary information about the excretion of ketone bodies as well as citrate, creatinine and other unidentified metabolites.

Finally we consider the origin of resonances attributable to mobile fatty acids in plasma, and investigate these in studies of subjects with non-insulin-dependent diabetes and lipid disorders, including a subject with hypertriglyceridaemia who responded to improved diabetic control.

Experimental

Serum, plasma and urine samples

Serum and urine were obtained (with consent) from normal and fasting male volunteers and placed into sterile containers. Blood was defibrinated as described previously (Nicholson *et al.*, 1983). In the fasting-subject experiments, blood and urine samples were taken at various times from the 25-year-old male volunteer who drank only water. ^2H_2O was added (usually 50μl to 0.45ml) prior to n.m.r. measurements.

For convenience, blood samples from diabetic subjects were collected into lithium heparin (anticoagulant) vials. Plasma was obtained after immediate removal of cells by centrifugation (5000*g*; 5min) at 4°C and stored frozen on solid CO_2 until measurement approx. 6h later. N.m.r. measurements on plasma and urine (which was frozen in a similar manner) were completed within approx. 1h of thawing. The decomposition rate of acetoacetate in diabetic plasma was only approx. 10% in 6h at 20°C as measured by n.m.r.

The non-insulin-dependent diabetic subject was a 48-year-old male with a blood glucose level of 21mM and 1+ ketonuria by the Ames 'acetest' [see Henry, 1964 (page 934)]. The insulin-dependent subject was a 19-year-old female who was withdrawn from insulin at 04:00h. This subject was participating in a research project which had been approved by the Ethical Research Committee of St. Thomas' Hospital. Most n.m.r. samples thawed to give relatively clear solutions.

N.m.r. measurements

These were made on a Bruker WH400 spectrometer operating at 400.13MHz (9.4T), 298K. Samples (usually 0.5ml total) were contained in 5mm-outer-diameter tubes. Spectra from serum and plasma samples were obtained by using the Hahn (1950) spin-echo pulse sequence, 90°-τ-180°-τ-collect, with a τ value of 60ms, 90° pulse 9.5μs, 180° pulse 19μs. When quantitative measurements were required, the sequence was repeated 48 times each with a total delay of 5.8s (16384 computer points, acquisition time 1.7s, delay 4.1s). This gave reasonable signal-to-noise (S/N) ratios with little suppression of the intensities of slowly relaxing nuclei due to T_1 (spin-lattice relaxation time) effects. When rapid pulsing was employed (every second), the acetone and acetoacetate methyl signals decreased in intensity by about 20% relative to those of alanine and hydroxybutyrate. Chemical shifts were referenced to external sodium 3-(trimethylsilyl)-1-propanesulphonate (TSP). When present, acetone at 2.235p.p.m. was used as an internal standard.

In general it was advantageous to suppress the large H_2O signal by applying a secondary irradiation field at its resonance frequency. Higher spectrometer receiver gains could then be used giving much improved S/N ratios. Since the levels of protein in urine are very low, spectra could also be obtained in normal (single-pulse) mode. For quantitative measurements on urine, a 40° pulse was used with a total pulse recycle time of 5.8s.

Sodium 3-DL-hydroxybutyrate, lithium acetoacetate, sodium lactate, L-alanine and L-valine (all purchased from Sigma) for standard additions were added to n.m.r. samples as 10–50μl of 50 or 100mM solutions in ^2H_2O, giving 1–5mM increments of the substance of interest as appropriate. Corrections for dilution were applied.

To calibrate plasma samples from the insulin-withdrawal subject, standard additions of acetoacetate, butyrate, alanine and valine were made to the +2, +10 and +12h samples. All were measured with similar spectrometer conditions, and cross-checks were made between standardizations on the same sample and between different samples. All measurements agreed to within $\pm$10%, except that for acetoacetate, which was up to 100% greater when calibrated from acetoacetate additions than when calibrated from other standards. The final values for acetoacetate are based on butyrate. Peak u_2 (Fig. 1, probably *N*-acetyl protons) provided a useful internal standard for cross-checks between samples. Initially, integrations were made by weighing paper traces, but large errors can arise from the choice of phase and baseline of spin-echo spectra. In the present case the linewidths of peaks were similar in each spectrum, and peak heights were used for the final determinations.

Enzymic assays for 3-D-hydroxybutyrate and acetoacetate

Blood was deproteinized by the addition of 5% (w/v, final concn.) $HClO_4$ and stored at −20°C. For the plasma measurements, heparinized blood

was centrifuged and the plasma added to an equal volume of 10% $HClO_4$. Measurements of 3-D-hydroxybutyrate and acetoacetate were performed by using the enzymic method described by Williamson *et al.* (1962). Preparation of the samples was laborious, since the denatured protein had first to be removed by centrifugation and the acidic supernatant neutralized with 20% (w/v) KOH. The resultant precipitate of $KClO_4$ was removed by centrifugation and the assays were performed on the clear supernatant.

Other assays

Amino acids in plasma were determined on a Chromaspek J180 automatic amino acid analyser, and creatinine in plasma and urine by an automated procedure based on the method of Folin & Wu [see Henry, 1964 (p. 300)]. Free (non-esterified) fatty acids in plasma were measured fluorimetrically by the method of Carruthers & Young (1973).

Results and discussion

Two main problems hamper attempts to obtain well-resolved proton Fourier-transform n.m.r. spectra from untreated serum and plasma. These are the large signal from H_2O (approx. 55M), and the severe overlap of resonances, particularly the broad ones from high-M_r proteins such as albumin and immunoglobulins. We have recently demonstrated (Nicholson *et al.*, 1983) one approach to overcoming these problems using a high-field spectrometer (9.4T) and the Hahn (1950) spin-echo pulse sequence together with irradiation of the solvent resonance.

The intensities of the signals in ^1H s.e.f.t. spectra, I (at time 2τ), are described approximately by the following equation (see Brown & Campbell, 1980):

$$I(2\tau)=I(0)\exp\left(-\frac{2\tau}{T_2}-\frac{2D\gamma^2 G^2 \tau^3}{3}\right)F(J)$$

where $I(0)$ is the signal amplitude after the 90° pulse.

Thus $I(2\tau)$ is dependent on three factors: firstly the relaxation time, T_2: this, in turn, is related to mobility; resonances from nuclei with T_2 values short compared with 2τ (120ms in the present work) will not contribute to the spin-echo spectrum; secondly, molecular diffusion to a region of different applied field (field gradient G, diffusion coefficient D) during the period 2τ; and thirdly, homonuclear spin–spin coupling, the $F(J)$ term. If the sample is homogeneous, the term involving G can usually be neglected for τ values up to 100ms. For many protons, J is approx. 8Hz and, by choosing $\tau = 1/2J = 60$ms, singlets and triplets remain upright, whereas doublets are inverted.

The intensities of resonances have to be interpreted cautiously at this stage, because of T_2 relaxation and possible heterogeneity of samples. However, we have found that the spectra of serum and plasma from normal human subjects under similar physiological conditions are highly reproducible. Spectra from serum show a strong resemblance to those of plasma, except for the presence of the resonances of anticoagulant in the latter. EDTA is useful if measurements of Ca and Mg levels are also required (Nicholson *et al.*, 1983), but interferes with the N-CH_3 region. Heparin (a polysaccharide) was preferred in the present work, since it produced negligible interference at the levels used.

Fasting

Fig. 1 shows the ^1H n.m.r. profiles of the plasma of a normal subject who had fasted for 12, 37, and 48h. As expected, the intensity of the peaks for glucose gradually decreased and those for the ketone bodies increased. Particularly notable is the significant amount of acetone present.

Between 37 and 48h a dramatic decrease in intensities of the two peaks P_2 and P_3 occurred (Fig. 1). These peaks appear to arise predominantly from the mobile CH_3 and $(CH_2)_n$ groups respectively of fatty acids (Nicholson *et al.*, 1983). During starvation, the total level of non-esterified fatty acids in the plasma is known to increase rather than decrease (Cahill *et al.*, 1966). Resonances from these fatty acids, which are tightly bound and therefore immobilized, would be expected to be broad (Stein *et al.*, 1968) and not to contribute to the spin-echo spectra. Indeed we have not seen such signals in spin-echo spectra of isolated albumin containing bound fatty acids (J. R. Bales, C. J. Coulson & P. J. Sadler, unpublished work). Rapid molecular motion of a portion of fats in triacylglycerol droplets and mobile parts of membranes has been observed previously in ^1H n.m.r. studies of serum lipoproteins (Chapman *et al.*, 1969). In the present case, these may also be in chylomicrons. The pool of fatty acids seen by n.m.r. appears to be readily metabolizable. Further evidence that signals from chylomicrons, triacylglycerols and lipoproteins contribute to peaks P_2 and P_3 will be discussed below.

Since the protein content of urine is normally low, both single-pulse and spin-echo spectra were obtainable from the urine of the fasting subject. Prominent peaks were those of creatinine and the ketone bodies. These results encouraged us to study diabetic subjects.

Insulin-dependent diabetes (diabetes mellitus)

An insulin-dependent subject whose diabetes had been controlled by intravenous infusion was

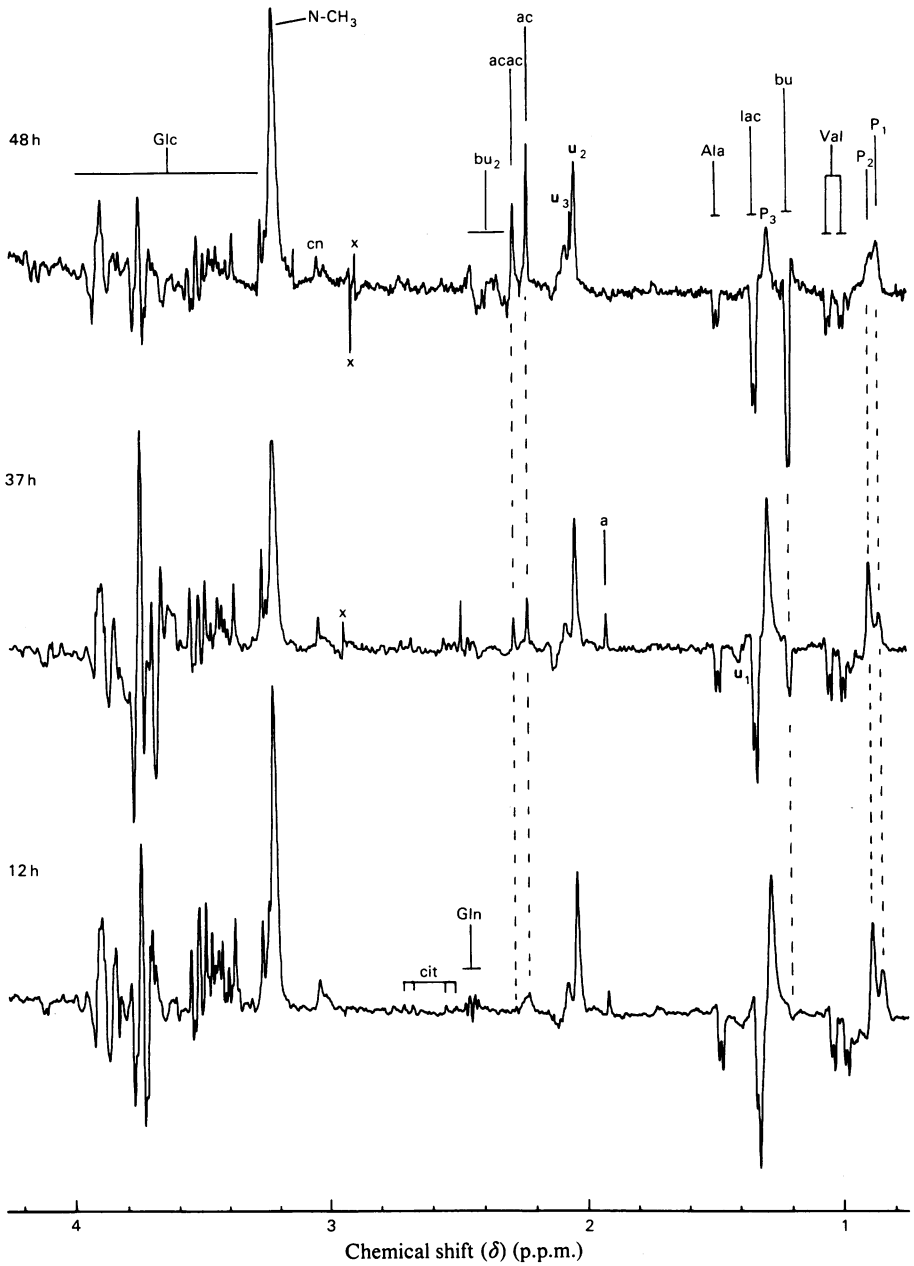

Fig. 1. *400 MHz s.e.f.t. ^1H n.m.r. spectra of serum samples from a subject who had fasted for 12, 37 and 48 h on separate occasions*

The spectra are the result of approx. 4.5 min accumulations. Note the gradual increase in intensity of resonances for the ketone bodies, the decrease in glucose and the sharp decrease in fatty-acid peaks P_2 and P_3 at 37 h. Noise 'spikes' are labelled 'x'. Assignments: P_2 and P_3, fatty acid CH_3 and $(CH_2)_n$ respectively; Val, valine CH_3; lac, lactate CH_3; Ala, alanine CH_3; Gln, glutamine CH_2; cit, citrate CH_2; cn, creatinine *N*-CH_3; Glc, glucose CH; bu and bu_2, 3-D-hydroxybutyrate CH_3 and CH_2 respectively; a, acetate; ac, acetone; acac, acetoacetate CH_3 groups; P_1, u_1, u_2 and u_3 are unknowns. Chemical shifts are referenced to trimethylsilapropane sulphonate (external). When present, acetone is a useful internal reference at 2.235 p.p.m.

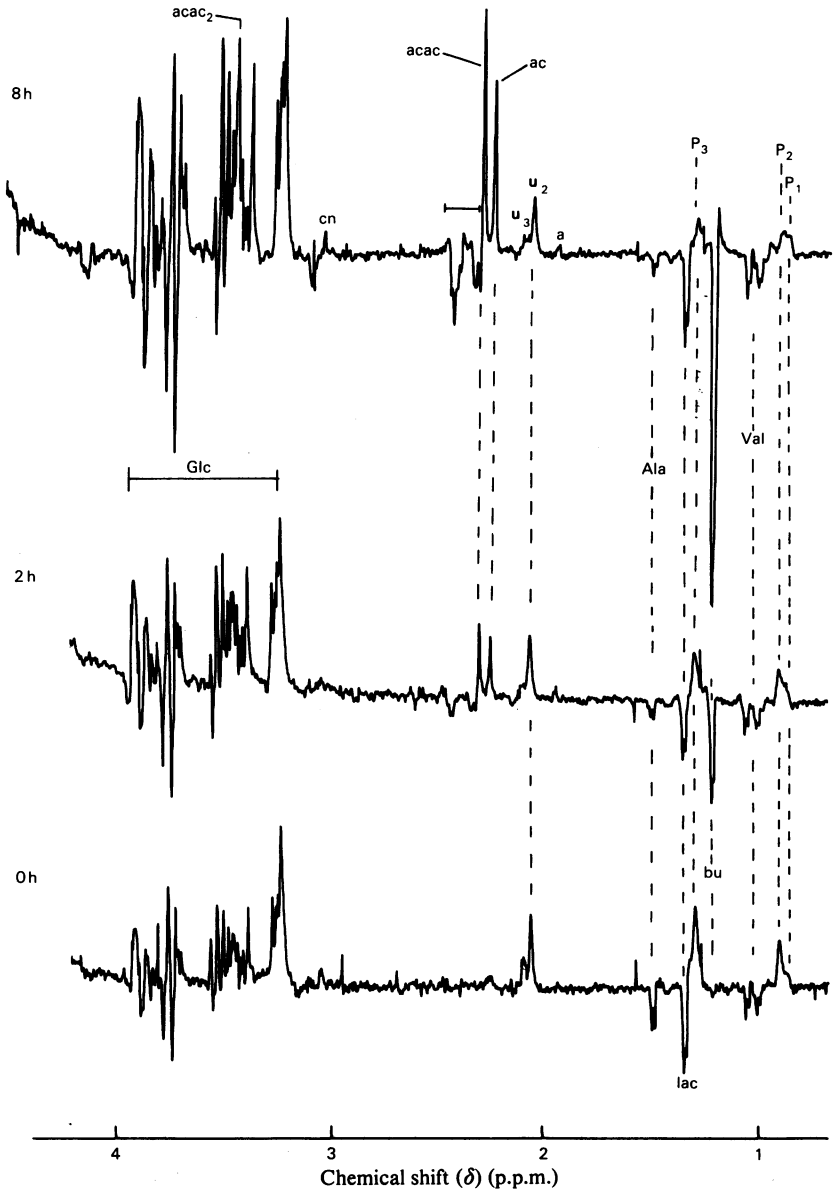

Fig. 2. *400 MHz spin-echo ^1H n.m.r. spectra of plasma samples from an insulin-dependent diabetic subject 0, 2, and 8 h after insulin withdrawal*

The progressive rise in glucose and ketone-body levels is seen. The marked decrease in intensity of peaks P_2 and P_3 assigned to fatty acids is comparable with that seen in Fig. 1. Assignments: $acac_2$, acetoacetate CH_2 resonance; for others, see Fig. 1.

withdrawn from this treatment at 04:00h, hereafter referred to as 'zero time'. Blood and urine samples taken at −1, 0, 2, 4, 6, 8, 10 and 12h were analysed by n.m.r. and selected ones by independent chemical assays.

At the point of insulin withdrawal the spectrum from plasma (Fig. 2) shows a strong resemblance to that of normal subjects. Subsequent spectra after withdrawal showed an elevation of glucose, 3-D-hydroxybutyrate, acetone and acetoacetate levels. The concentration of the ketone bodies, lactate, alanine and valine were determined by integration of methyl peaks at 1.43, 1.49, and 1.05p.p.m. respectively in spin-echo spectra before and after

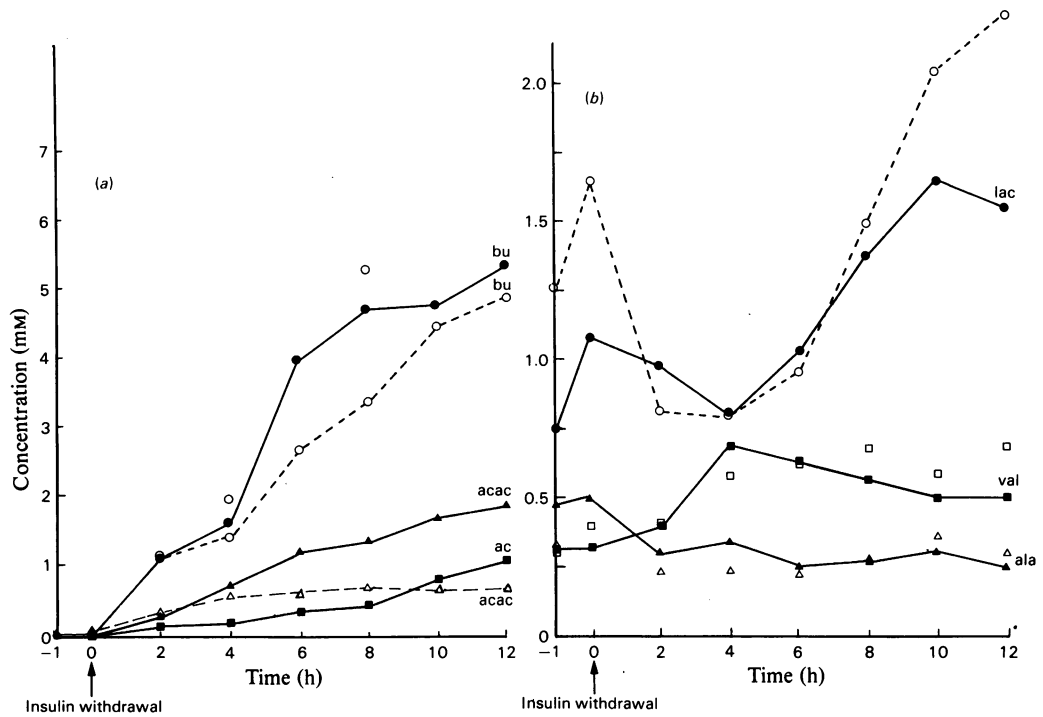

Fig. 3. *A comparison of the concentrations of 3-D-hydroxybutyrate (bu), acetoacetate (acac), acetone (ac), lactate (lac), valine (Val) and alanine (Ala) in plasma samples from an insulin-dependent diabetic subject at various times after insulin withdrawal as determined by n.m.r. (closed symbols) and conventional assays (open symbols)*

The broken lines for acac, bu and lac refer to measurements on whole blood; all other symbols refer to plasma samples. Measurements of acac in plasma *by conventional assay* at −1, +2 and + 6h gave values the same as those shown *for whole blood.*

standard additions. The results are shown in Fig. 3, where they are compared with those obtained by standard enzymic assays for 3-D-hydroxybutyrate, acetoacetate and lactate, and with determinations of valine and alanine on an amino acid analyser.

With the exception of acetoacetate, the agreement is remarkably good. The level of hydroxybutyrate rises from near zero to approx. 5mM in 12h after insulin withdrawal. The concentration of the glucogenic amino acid alanine decreased progressively, whereas that of valine, a ketogenic amino acid, increased (Fig. 3). The pattern of lactate levels measured by n.m.r. shows a reasonable agreement with those by enzymic assays of whole blood. The n.m.r. resonances for these metabolites therefore appear to account for most of the total amounts of them present in the samples.

The discrepancy for acetoacetate is marked. By enzymic assay the level reached a peak at approx. 0.7mM, whereas by n.m.r. a progressive rise to approx. 2mM was observed (Fig. 3). Acetoacetate is formed from acetyl-CoA in three steps, and its conversion into 3-D-hydroxybutyrate is catalysed by 3-D-hydroxybutyrate dehydrogenase in mitochondria. Spontaneous decarboxylation of acetoacetate produces acetone. Progressive increases in the ratio of hydroxybutyrate to acetoacetate concentrations from approx. 1.5 to greater than 6 after 10–12h of insulin withdrawal have been reported previously (Barnes *et al.*, 1977). This is generally considered to result from the increased NADH/NAD^+ ratio as the pH decreases inside mitochondria during ketosis. As measured by n.m.r. the ratio remained relatively constant at 3.0 ± 0.2 during insulin withdrawal.

A second insulin-dependent diabetic subject was examined during the course of insulin withdrawal and results parallel with those described above were obtained. In this case the acetoacetate concentration by enzyme assay reached a plateau at approx. 0.5mM, whereas by n.m.r. it rose to approx. 2.3mM after 13h of withdrawal.

The calibration of acetoacetate levels by n.m.r. presented a problem. Initially peak areas together with acetoacetate 'standard' additions were used, but this gave overestimates of up to 600% compared with the enzymic assay. Much of this error was found to be associated with the choice of phase

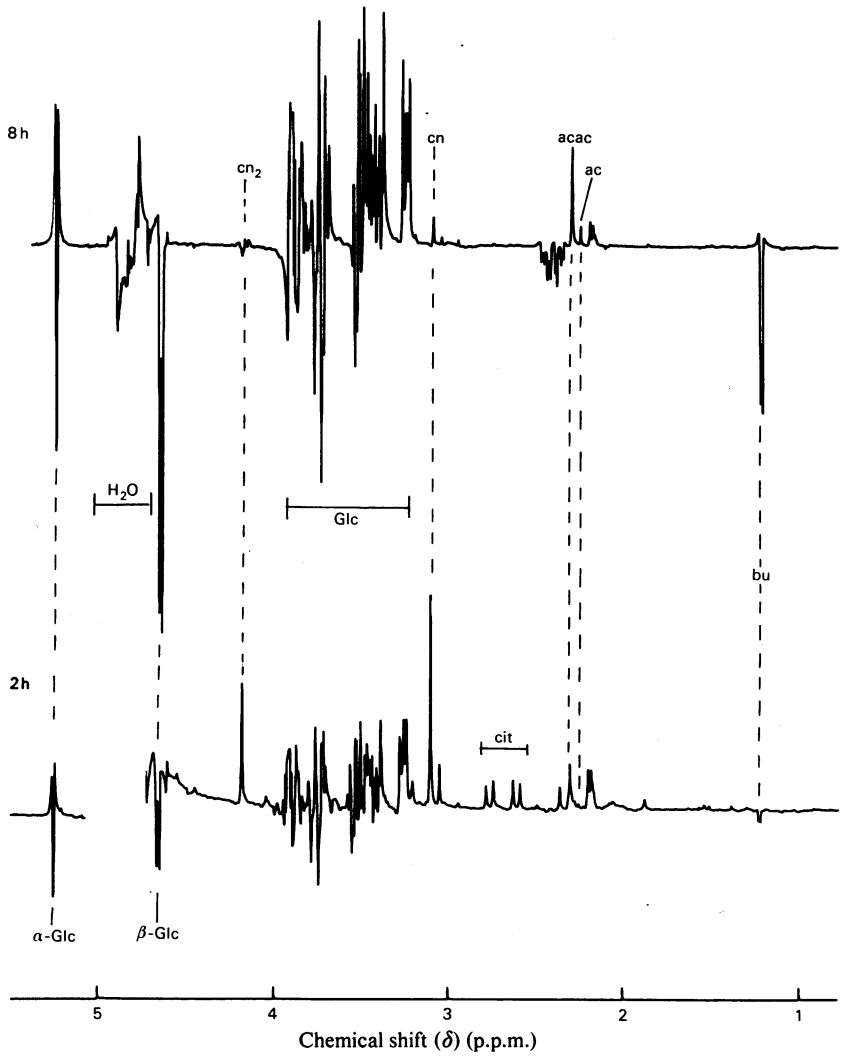

Fig. 4. *400 MHz spin-echo ^1H n.m.r. spectra of urine samples taken at +2 and +8 h after insulin withdrawal from a diabetic human subject*

For assignment labels, see Fig. 1; α-Glc, β-Glc and Glc are the anomeric and ring-proton resonances of glucose. The glucose levels were not calibrated, but can be seen to rise from approx. 40 mM at +2 h to more than 130 mM at +8 h.

and baseline of the spin-echo spectra and the uncertain purity (especially water content) of commercially available lithium acetoacetate. A further error (approx. 10%) arises from overlap with a wing of the CH_2 multiplet of butyrate, which is upright even though most of the multiplet is inverted at $\tau = 60$ ms. The CH_2 protons of acetoacetate are acidic and undergo ^1H–^2H exchange in ^2H_2O solution (Grande & Rosenfeld, 1980). Since we are irradiating the solvent resonance, we investigated the possibility that a positive enhancement of the acetoacetate CH_3 resonance was occurring via an n.O.e. (see Jardetsky & Roberts, 1982). However, in water, less than 2% exists in the enol form (Grande & Rosenfeld, 1980), and for lithium acetoacetate in $^1H_2O/^2H_2O$ (9:1, v/v) we found that the n.O.e. amounted to only +7%. No correction for this has been applied to our data. Our searches of the literature have revealed very few studies of the effects of pH and metal ions on the stability of acetoacetate and the position of the keto–enol equilibrium in aqueous (especially ^1H_2O) solutions. The current work suggests that further studies are worth while. We have found no evidence for acetone or hydroxybutyrate interference in the enzyme assay, but the possibility that a specific in-

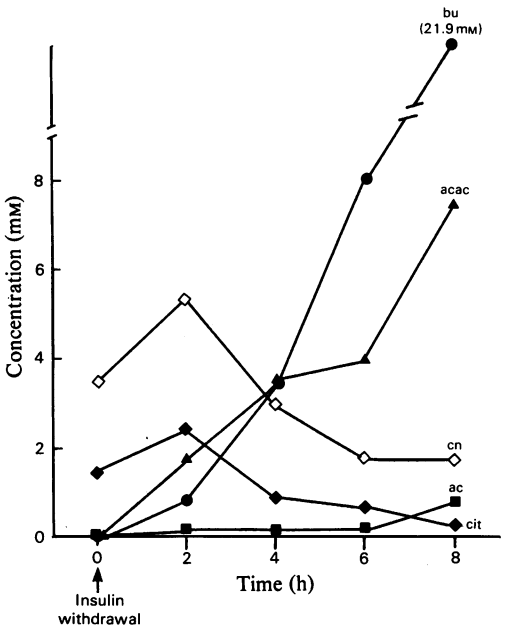

Fig. 5. *Dependence of the urine concentrations of 3-D-hydroxybutyrate (bu), acetoacetate (acac), creatinine (cn), acetone (ac) and citrate (cit) on time after insulin withdrawal from a diabetic subject*

The estimates are based on peak heights of normal proton spectra relative to creatinine as determined by a chemical assay.

hibitor is present in ketotic diabetic blood cannot be ruled out.

The concentrations of acetoacetate observed in urine by ^1H n.m.r. were comparable with those in plasma. Since the protein content of urine is low, well-resolved spectra were obtainable by using single pulses together with water irradiation, although spin-echo spectra were useful for assignment purposes. Typical spectra are shown in Fig. 4. The high level of glucose (>100mM) is evident, together with peaks for 3-D-hydroxybutyrate, acetoacetate, creatinine, acetone, citrate and several other metabolites that have not yet been identified.

No standard additions were made to urine samples, but the data were analysed by comparing peak heights (normal spectra) with those of creatinine for which the concentrations determined by independent assays were assumed. The results are shown in Fig. 5. The paralleling of creatinine and citrate excretion is notable. The acetoacetate/acetone ratio in urine decreases progressively from 22 to 9 between 2 and 8h after insulin withdrawal. The difference between this and the ratio in plasma suggests that there are different renal-tubular reabsorption processes for these two metabolites. The hydroxybutyrate/acetoacetate ratio gradually increases from 0.5 at +2h to 3.0 at +8h after withdrawal, a value similar to that found in plasma by n.m.r.

Fatty acids

During insulin withdrawal, the behaviour of the peaks P_2 and P_3 assigned to mobile CH_3 and $(CH_2)_n$ groups of fatty acids is notable (Fig. 2). They show a progressive decrease in intensity relative to, say, P_1, which remains constant, such that after 12h withdrawal the P_2/P_1 and P_3/P_1 ratios are very similar to those after 36h of fasting by the normal subject (approx. 1:1; compare Figs. 1 and 2). Thus the mobile pool of fatty acids seen by n.m.r. is rapidly metabolized by the diabetic subject after insulin withdrawal. In contrast, the concentration of the so-called 'free fatty acids', measured fluorimetrically, increased from 0.33 to 1.92mM 12h after withdrawal.

A dramatic elevation in the intensities of resonances P_2 and P_3 was seen in the spectrum of plasma from a 53-year-old obese non-insulin-dependent diabetic subject (Fig. 6). Other resonances for fatty acids are also intense and provide evidence of the presence of unsaturation (P_8, e.g. oleate, linoleate). The spectrum closely resembles that of plasma from a capybara (*Hydrochoerus hydrochaeris*) which we reported previously (Nicholson *et al.*, 1983).

Plasma samples from several subjects with disorders of lipid metabolism, such as familial hypertriglyceridaemia, showed dramatic elevations of the intensities of peaks P_2–P_8 (J. K. Nicholson, P. J. Sadler, A. F. MacLeod, S. M. Juul & P. H. Sönksen, unpublished work). Taken together, these studies suggest that the fatty acids which give rise to n.m.r. signals are in chylomicrons and lipoproteins.

In Fig. 7, proton n.m.r. spectra of a diabetic subject with hypertriglyceridaemia and Type IV hyperlipoproteinaemia are shown. The decrease in the level of n.m.r.-detectable fatty acids as he responded to insulin therapy is clearly evident.

Comparison of n.m.r. and other assays

The major advantages of n.m.r. over other assays are that little or no pretreatment of the samples is required and that information about a range of metabolites can be obtained in a single experiment. In most other assays the choice of substrate has to be made before the determination begins. With n.m.r., all mobile protons at millimolar levels are scanned simultaneously. This can provide unexpected information. For example, we found inverted multiplets at approx. 2.8–3.0 p.p.m. in spectra from plasma of the insulin-withdrawal

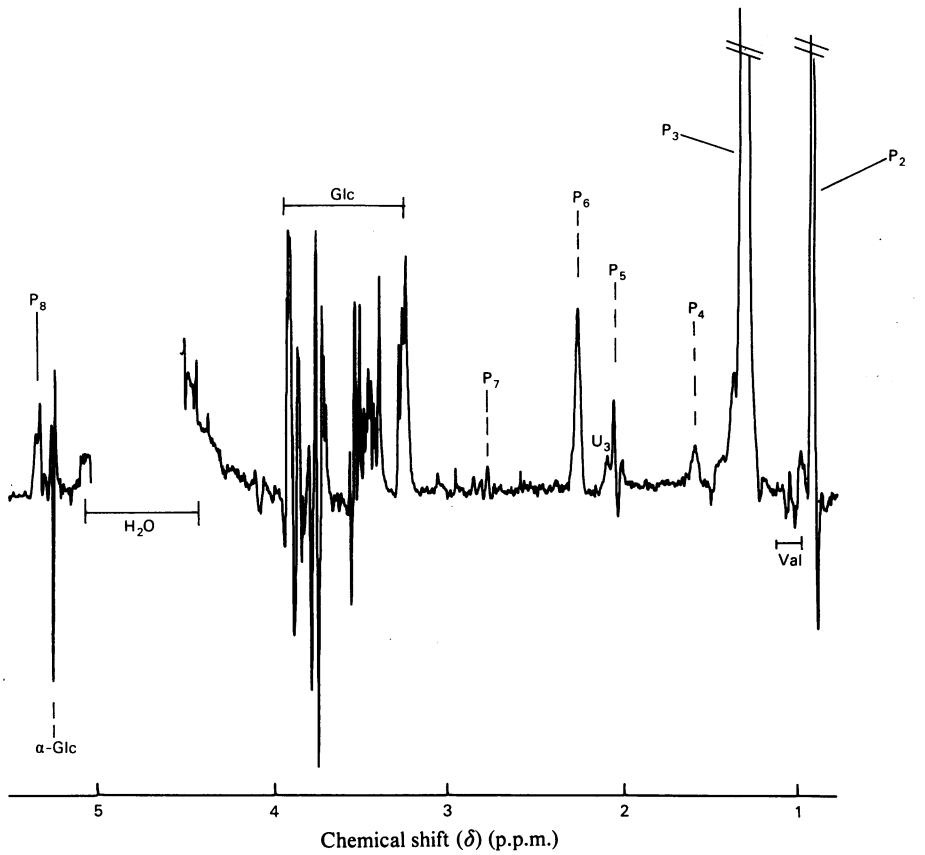

Fig. 6. *400 MHz spin-echo ^1H n.m.r. spectrum (296 scans, 8192 points, 1 s total delay between pulses) of a plasma sample from a non-insulin-dependent diabetic human subject*

Most prominent are the intense peaks assignable to unsaturated fatty acids: P_2, $\underline{CH_3}$; P_3, $\underline{(CH_2)_n}$; P_4, $\underline{CH_2}CH_2CO$; P_5; $CH{=}CH\underline{CH_2}$; P_6, $\underline{CH_2}CO$–; P_7, $C{=}CH{-}\underline{CH_2}{-}CH{=}C$; P_8, $\underline{CH}{=}\underline{CH}$.

subject at −1 and +4h. These have not yet been identified.

For many chemical assays the preparation of samples is tedious, often involving acidification, precipitation of proteins and neutralization. However, such assays often give accurate and reliable results, since interference problems have been solved during the course of many years of usage. The extent of interferences in the n.m.r. determinations is as yet unclear. Possible complications can arise during spin-echo spectroscopy from the cancellation of positive and negative overlapping peaks and variations in relaxation rates. It is often possible to check these, since the signals for ketone bodies are clearly visible on top of the protein envelope in normal (single-pulse) spectra. However, 'n.m.r.-invisible' pools of metabolites can still exist if they are bound to macromolecules or paramagnetic metal ions. The ^1H n.m.r. profiles for the 20 or so diabetics that we have examined so far all gave reliable indications of the disease state. We do not know of any other single method which can provide an assay of all the three major ketone bodies in plasma and urine without sample pretreatment.

N.m.r. is of course of little value for metabolites which do not have detectable protons, or those present at low concentration (<approx. 0.1 mM), a situation which is exacerbated if the protons form part of a highly coupled spin system (complex multiplets). By amino acid analysis on an autoanalyser 16 amino acids were detected in a typical sample of diabetic plasma at concentrations ranging from 0.01 to 0.5mM (4h after withdrawal): Asp, 0.01; Thr, 0.09; Ser, 0.01; Glu, 0.17; Gln, 0.39; Gly, 0.17; Ala, 0.19; Cys, 0.06; Val, 0.46; Ile, 0.13; Leu, 0.26; Tyr, 0.06; Phe, 0.06; His, 0.07; Orn, 0.07; Lys, 0.14mM. Only two of these amino acids, valine and alanine, could be readily determined by n.m.r. However, n.m.r. should not be

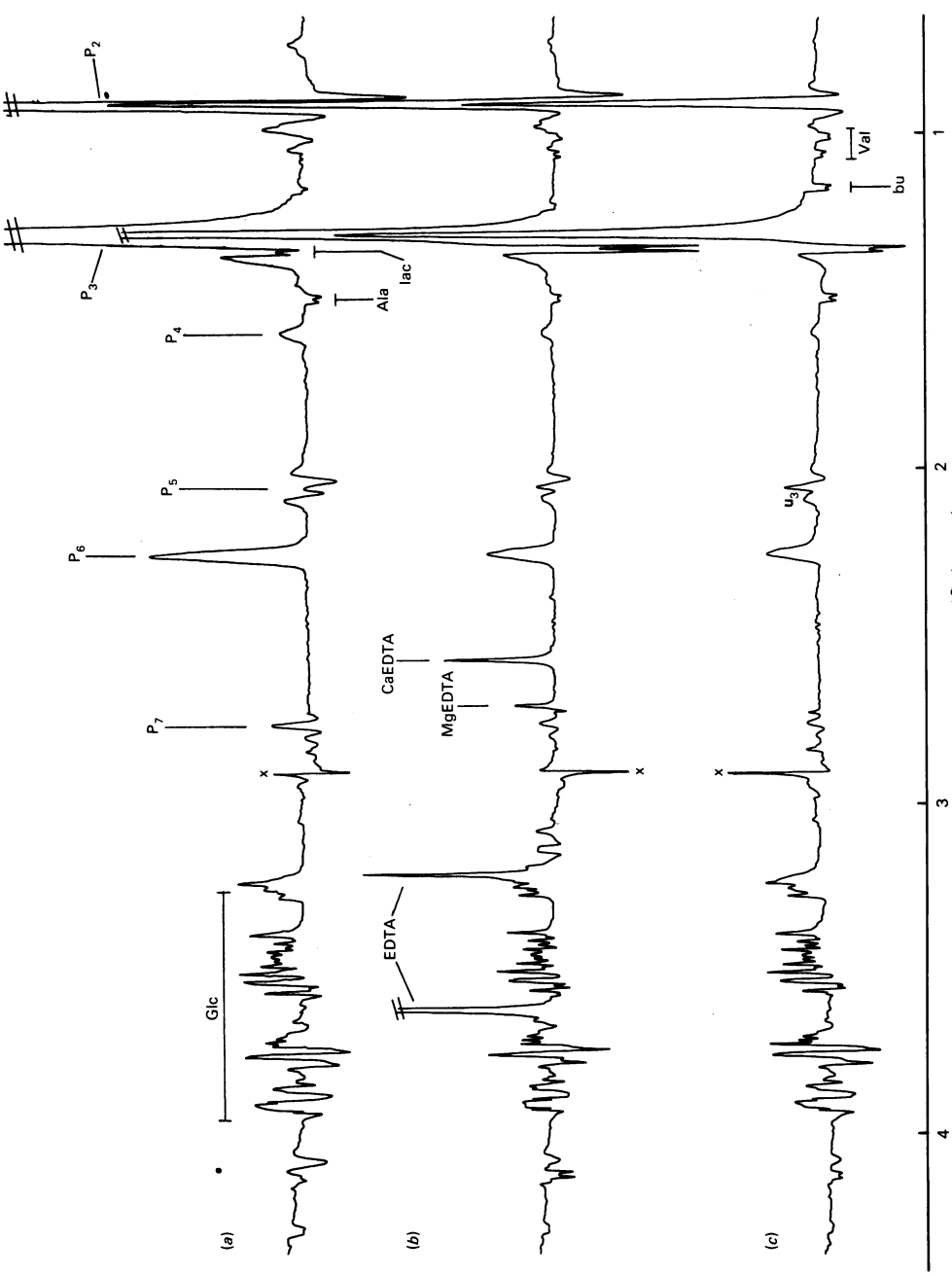

Fig. 7. *400 MHz spin-echo* ^1H *n.m.r. spectra of plasma samples from a diabetic subject with hypertriglyceridaemia*

(*a*) Poor control of diabetes; (*b*) and (*c*) 4 and 10 days respectively after modification of insulin therapy and diet to achieve better diabetic control. Note the gradual return toward normality of the intensities of the fatty acid peaks P_2–P_8 (for assignments, see Fig. 6). Samples for (*a*) and (*c*) were collected into heparinized containers, whereas for (*b*) K_2EDTA was used as anticoagulant, (*b*) therefore has peaks for free EDTA, $CaEDTA^{2-}$ (approx. 2.5 mM), and $MgEDTA^{2-}$ (approx. 1 mM).

viewed simply as a quantitative assay. Spectra also contain information about the mobilities of molecules and their interactions. An n.m.r. fingerprint of a metabolic disorder such as diabetes is likely to be unique. In the case of diabetes, our results suggest that n.m.r. can be useful in its diagnosis and treatment. This may also be true of a range of other diseases.

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References

Barnes, A. J., Bloom, S. R., Alberti, K. G. M. M., Smythe, P., Alford, F. P. & Chisholm, D. J. (1977) *N. Engl. J. Med.* **269**, 1250–1253

Bock, J. L. (1982) *Clin. Chem.* **28**, 1873–1877

Brown, F. F. & Campbell, I. D. (1980) *Proc. R. Soc. London Ser. B* **289**, 395–406

Cahill, G. F., Herrera, M. G., Morgan, A. P., Soeldner, J. S., Steinke, J., Levy, P. L., Reichard, G. A. & Kipnis, D. M. (1966) *J. Clin. Invest.* **45**, 1751–1769

Carruthers, M. E. & Young, D. A. (1973) *Clin. Chim. Acta* **49**, 341–348

Chapman, D., Leslie, R. B., Hirz, R. & Scanu, A. M. (1969) *Biochim. Biophys. Acta* **176**, 524–536

Daniels, A., Williams, R. J. P. & Wright, P. E. (1976) *Nature (London)* **261**, 321–323

Grande, K. D. & Rosenfeld, S. M. (1980) *J. Org. Chem.* **45**, 1626–1628

Hahn, E. L. (1950) *Phys. Rev.* **80**, 580

Henry, R. J. (1964) *Clinical Chemistry: Principles and Technics*, Hoeber Medical Division, Harper and Row, New York

Iles, R. A., Buckingham, M. J. & Hawkes, G. E. (1983) *Biochem. Soc. Trans.* **11**, 374–375

Jardetsky, O. & Roberts, G. C. K. (1982) *NMR in Molecular Biology*, Academic Press, New York

Nicholson, J. K., Buckingham, M. J. & Sadler, P. J. (1983) *Biochem. J.* **211**, 605–615

Rabenstein, D. L. & Nakashima, T. T. (1979) *Anal. Chem.* **51**, 1465A–1474A

Stein, J. M., Edner, O. J. & Bargoot, F. G. (1968) *Science* **16**, 909–911

Williamson, D. H., Mellanby, J. & Krebs, H. A. (1962) *Biochem. J.* **82**, 90–96