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Propionate metabolism in the rat heart by ^{13}C n.m.r. spectroscopy

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High-resolution ^{13}C n.m.r. spectroscopy has been used to examine propionate metabolism in the perfused rat heart. A number of tricarboxylic acid (TCA) cycle intermediates are observable by ^{13}C n.m.r. in hearts perfused with mixtures of pyruvate and propionate. When the enriched ^{13}C-labelled nucleus originates with pyruvate, the resonances of the intermediates appear as multiplets due to formation of multiply-enriched ^{13}C-labelled isotopomers, whereas when the ^{13}C-labelled nucleus originates with propionate, these same intermediates appear as singlets in the ^{13}C spectrum since entry of propionate into the TCA cycle occurs via succinyl-CoA. An analysis of the isotopomer populations in hearts perfused with [3-^{13}C]pyruvate plus unlabelled propionate indicates that about 27% of the total pyruvate pool available to the heart is derived directly from unlabelled propionate. This was substantiated by perfusing a heart for 2 h with [3-^{13}C]-propionate as the only available exogenous substrate. Under these conditions, all of the propionate consumed by the heart, as measured by conventional chemical analysis, ultimately entered the oxidative pathway as [2-^{13}C] or [3-^{13}C]pyruvate. This is consistent with entry of propionate into the TCA cycle intermediate pools as succinyl-CoA and concomitant disposal of malate to pyruvate via the malic enzyme. ^{13}C resonances arising from enriched methylmalonate and propionylcarnitine are also detected in hearts perfused with [3-^{13}C] or [1-^{13}C]propionate which suggests that ^{13}C n.m.r. may be useful as a non-invasive probe *in vivo* of metabolic abnormalities involving the propionate pathway, such as methylmalonic aciduria or propionic acidaemia.

INTRODUCTION

Propionate derived from odd-chain carbon fatty acids or degradation of certain amino acids is efficiently metabolized by the heart. The propionate pathway, reviewed in Fig. 1, involves an initial carboxylation of propionyl-CoA to form methylmalonyl-CoA followed by an isomerization to succinyl-CoA. This is considered an anaplerotic pathway since it leads directly to a tricarboxylic acid (TCA) cycle intermediate. Further oxidation of propionate requires subsequent degradation of a TCA cycle intermediate into a C3 compound that may re-enter the cycle as acetyl-CoA. Recent results have shown that the NADP-dependent malic enzyme is active during propionate utilization (Hiltunen & Davis, 1981). If this is the major degradation pathway for removal of TCA cycle intermediates, propionate carbons may ultimately add to the pyruvate pool available to pyruvate dehydrogenase (PDH). ^{14}C results from perfused rat hearts indicate that propionate does indeed contribute to the pyruvate pool, but estimates of the percentage contribution range from a low of 20% (Sundqvist *et al.*, 1984) to a high of 100% (Latipaa *et al.*, 1985). This large disparity reflects the uncertainty in measuring the flux through PDH using conventional radiolabelling techniques (Latipaa *et al.*, 1985).

We have recently developed a ^{13}C n.m.r. technique for measuring relative fluxes and fractional enrichments of labelled molecules entering the TCA cycle of the heart (Malloy *et al.*, 1987, 1988). In this work, the technique has been used to examine the fate of ^{13}C-enriched propionate in the perfused rat heart. The results indicate that 27% of the pyruvate pool available to PDH is derived from propionate in hearts perfused with equimolar mixtures of pyruvate and propionate and this increases to about 40% in hearts perfused with propionate as the sole exogenous substrate.

METHODS

Materials

[3-^{13}C]Sodium propionate (91.1%) and [3-^{13}C]sodium pyruvate (99.6%) were purchased from MSD Isotopes (St. Louis, MO, U.S.A.). Sodium [*Me*-^{13}C]methylmalonate was prepared from $^{13}CH_3I$ (MSD Isotopes) and diethylmalonate using published procedures (Blatt, 1943). The product was judged to be 98% pure by ^{1}H and ^{13}C n.m.r., the major impurity being the starting material, diethylmalonate. The ester was hydrolysed in aqueous base and [*Me*-^{13}C]methylmalonate isolated as the sodium salt.

Perfusions

Rat hearts were perfused by the Langendorff method using an all glass water-jacketed, perfusion apparatus which fits entirely into the bore of an 89 mm 7 T magnet.

Abbreviations used: TCA, tricarboxylic acid; PDH, pyruvate dehydrogenase.

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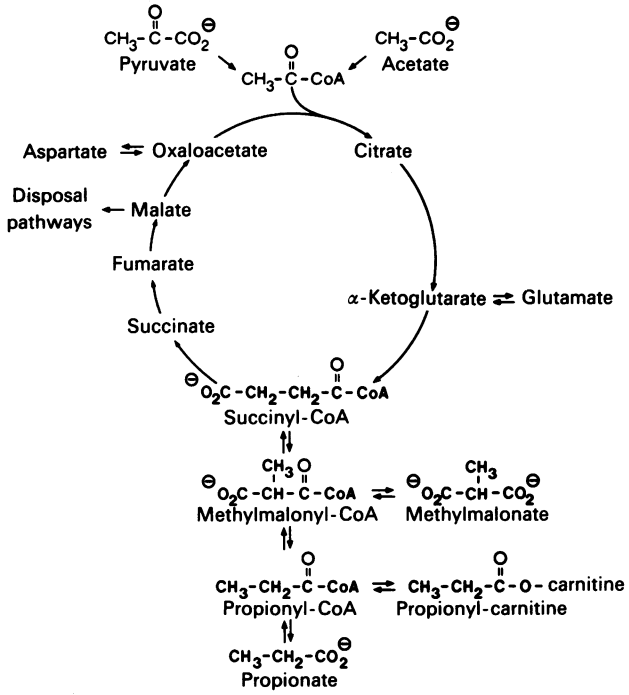

Fig. 1. The metabolic pathway for entry of propionate into the TCA cycle

The temperature was maintained at 37 °C throughout. A standard Krebs–Henseleit medium containing 1.2 mM-phosphate, 2.5 mM-Ca^{2+} and the ^{13}C-enriched substrate (no glucose or insulin) was bubbled continuously with a 95% O_2/5% CO_2 mixture at a column height of 70 cm. Hearts were excised and attached to the apparatus within 30 s to prevent ischaemia. Approx. 100 ml of perfusate was recirculated with continuous filtration throughout the n.m.r. experiment. Hearts were allowed to beat spontaneously and the rate was monitored using a water filled line running from the left ventricle to a pressure transducer external to the magnet.

N.m.r. spectra

^{13}C n.m.r. spectra were recorded on a Nicolet NT 300 spectrometer using the MLEV16 sequence for broad-band proton decoupling. Each heart was perfused to steady-state ^{13}C-enrichment while monitoring the ^{13}C n.m.r. spectrum with time as described previously (Malloy *et al.*, 1988). After obtaining a final steady-state ^{13}C spectrum of a heart in the magnet, the tissue was freeze-clamped using standard techniques, extracted into a cold perchloric acid solution, neutralized, and freeze-dried. The dry powder was dissolved into deuterated water, adjusted to pH 7.4, and scanned in a 10 mm probe under high-resolution conditions.

^{13}C isotopomer analysis

The multiplets observed in the ^{13}C spectrum of a heart perfused to steady-state with [3-^{13}C]pyruvate (or an equivalently labelled acetyl-CoA source) reflect both the ^{13}C-fractional enrichment of all molecules entering the TCA cycle pools and the relative flux of carbon through the combined anaplerotic pathways versus that through citrate synthase (Malloy *et al.*, 1987, 1988). The individual component areas may be derived from an observed multiplet to obtain the desired flux information using the equations presented below. The complete derivation of these equations has been presented elsewhere (Malloy *et al.*, 1988).

$$C2S = [2(y+1)^2 - 2F_{c2}(y+1) - F_{c2} + (F_{c2})^2]/[2(y+1)^2] \quad (1)$$

$$C2D12 = F_{c0}F_{c2}/[2(y+1)^2] \quad (2)$$

$$C2D23 = F_{c2}(2+2y-F_{c2})/[2(y+1)^2] \quad (3)$$

$$C3S = F_{c0}(F_{c0}+y)/(y+1) \quad (4)$$

$$C3T = (F_{c2})^2/(y+1) \quad (5)$$

$$C4D34 = (yF_{a1}+F_{c2})/(2y+1) \quad (6)$$

C2S refers to the area of the C-2 singlet relative to the total area of the C-2 multiplet, C2D12 refers to the area of the doublet resulting from spin–spin coupling between C-1 and C-2 (J_{12}) relative to the total area of the C-2 multiplet, etc. Since $J_{23} \neq J_{12}$ in glutamate, a second doublet contributes to the multiplet and its area is given as C2D23. The remaining multiplet component areas are similarly defined. The relative areas of the multiplets were measured from the ^{13}C n.m.r. spectrum by peak integration using the NMC software, by triangulation (peak height × width at half-height) and by plotting and weighing each resonance. Three independent variables (F_{c2}, y and F_{a1}) were evaluated from the n.m.r. data by a non-linear least-squares fit of equations 1–6 using a simplex routine which runs on an IBM-PC. F_c refers to the fractional enrichment in ^{13}C of the acetyl-CoA pool which condenses with oxaloacetate to form citrate and F_{c0} indicates the fraction of the acetyl-CoA pool that is unlabelled. Under the present perfusion conditions, with [3-^{13}C]pyruvate as the sole exogenous labelled substrate, $F_{c0}+F_{c2}=1$. If precursors which yield C-1-enriched acetyl-CoA are available to the heart, another fractional enrichment term is included (F_{c1}) and a more complete set of equations is necessary (Malloy *et al.*, 1988). The second variable, y, relates the total flux through the anaplerotic reactions (a) to the total flux through citrate synthase (c), or $y = a/c$ (Malloy *et al.*, 1987, 1988). Since the concentrations of the TCA cycle intermediates are not changing in steady-state, it was assumed that the pathways for removal of carbon skeletons are as active as those for entrance of carbon skeletons. The third group of variables refers to the ^{13}C-labelling of carbon skeletons entering the TCA cycle via the various anaplerotic pathways. F_{a0} indicates the fraction of anaplerotic substrate that is unlabelled and F_{a1} is the fraction of substrate that will yield either [2-^{13}C]oxaloacetate or [3-^{13}C]oxaloacetate in the first span of the TCA cycle. By definition, $F_{a1}+F_{a0}=1$. Notice that the parameter F_{a1} only appears in eqn. 6, so even though each of the component areas is sensitive to flux through the anaplerotic pathways (y), C4D34 is the only component area which changes when an anaplerotic precursor molecule contains an enriched-^{13}C nucleus.

RESULTS

Fractional enrichment and flux in pyruvate perfused hearts

^{13}C spectra of rat hearts perfused with [3-^{13}C]pyruvate as the sole exogenous substrate have been reported previously (Sherry *et al.*, 1985; Malloy *et al.*, 1987,

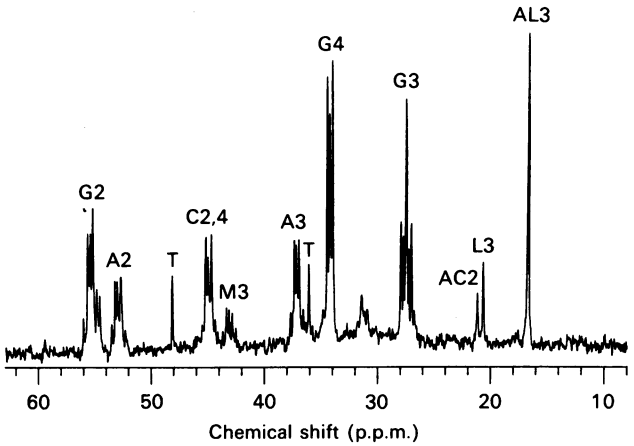

Fig. 2. ^{13}C n.m.r. spectrum of the extract of a rat heart perfused to steady-state with 2.5 mM-[3-^{13}C]pyruvate as the sole exogeneous substrate

The letter above each resonance designates the enriched metabolite and the number reflects the carbon position within that metabolite. G is glutamate, M is malate, A is aspartate, C is citrate, L is lactate, AL is alanine, AC is acetylcarnitine, T is resonances from natural abundance taurine.

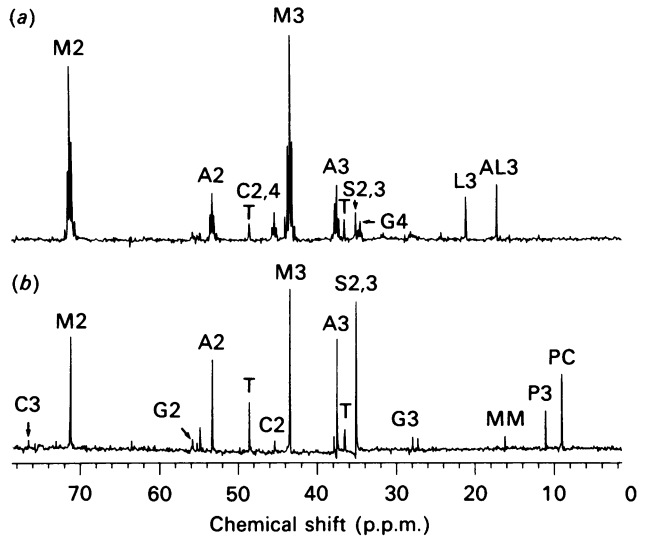

Fig. 3. ^{13}C n.m.r. spectra of extracts of hearts perfused to steady-state with 2.5 mM-[3-^{13}C]pyruvate plus 2 mM-unlabelled propionate (*a*) or 2.5 mM-unlabelled pyruvate plus 2 mM-[3-^{13}C]propionate (*b*)

The resonances are labelled as outlined in the legend to Fig. 2; in addition, S is succinate, P is propionate, MM is the methyl resonance of methylmalonate, PC is the methyl resonance of propionylcarnitine.

1988). A high-resolution spectrum of an extract of a [3-^{13}C]pyruvate-perfused heart is shown in Fig. 2. Several resonances appear as multiplets [Glu C-2, C-3 and C-4; citrate C-2 and C-4; Asp C-2 and C-3; and malate C-2 (not shown) and C-3], while others are clearly singlets [Ala C-3; lactate C-3; acetylcarnitine C-2; and taurine C-1 and C-2 (natural abundance)]. The metabolites whose resonances are multiplets are either TCA cycle intermediates or are formed directly from a cycle intermediate (i.e. glutamate from α-ketoglutarate). The multiplets arise from scrambling of ^{13}C in the TCA cycle to form mixtures of ^{13}C isotopomers in each intermediate pool (Chance *et al.*, 1983; Sherry *et al.*, 1985; Malloy *et al.*, 1987, 1988). We have shown that the relative singlet, doublet, triplet and quartet component areas of each multiplet reflects ^{13}C fractional enrichment of acetyl-CoA entering the TCA cycle and the relative flux through the combined anaplerotic pathways versus flux through citrate synthase (Malloy *et al.*, 1988). For example, the equations presented in the Methods section may be applied to the spectrum in Fig. 2; the analysis for this heart indicates that 92% of the acetyl-CoA entering the TCA cycle was derived from [3-^{13}C]pyruvate (via PDH), and the total flux through all combined anaplerotic reactions involving TCA cycle intermediates was 18% of the flux through citrate synthase. The analysis also indicates that very little [3-^{13}C]pyruvate entered the TCA cycle pools via direct carboxylation (i.e. $F_{a1} \cong 0$). The source of the unlabelled carbons which entered via an anaplerotic reaction cannot be determined from these results.

Substrate competition in pyruvate plus propionate perfused hearts

If unlabelled propionate were to be added as a second exogenous substrate, one would expect *a priori* that the anaplerotic flux should increase due to entry of unenriched propionate carbons into the TCA cycle intermediate pools (Fig. 1), and that the percentage of ^{13}C in the pyruvate pool available to PDH would decrease only if those propionate carbons which entered the cycle ultimately end up as pyruvate via a disposal reaction. A typical ^{13}C spectrum of an extract of a rat heart which had been perfused to steady-state with a mixture of [3-^{13}C]pyruvate and unenriched propionate is shown in Fig. 3(*a*). There are two major differences between this spectrum and the one shown in Fig. 2. First, the steady-state concentrations of succinate, fumarate (resonance at 136 p.p.m. not shown), malate and aspartate all increase while the glutamate and alanine levels decrease. These changes in metabolite concentrations parallel those measured in glucose versus glucose plus propionate-perfused hearts using conventional enzymatic methods (Sundqvist *et al.*, 1984). Secondly, the ^{13}C enrichment at glutamate C-2 and C-3 is substantially lower than at glutamate C-4 in the [3-^{13}C]pyruvate plus propionate perfused hearts. This is consistent with entry of natural abundance propionate carbons into the four carbon TCA cycle intermediate pools which dilutes the ^{13}C enrichment at glutamate C-2 and C-3, but not at C-4 (Malloy *et al.*, 1987, 1988). We have also observed that the intensity of the succinate resonance at 35 p.p.m. is quite variable from one heart to another, apparently due to small differences (~ 5%) in the amount of propionate added to each perfusate. Although the glutamate C-2 and C-3 are barely detectable in this spectrum (Fig. 3*a*), the malate or aspartate C-2 singlet and doublet intensities may be measured and, together with the glutamate C-4 singlet and doublet intensities used to analyse the fractional enrichment and flux ratio as outlined above (Malloy *et al.*, 1988). This analysis for a [3-^{13}C]pyruvate plus propionate-perfused heart indicates that 65% of the acetyl-CoA which entered the TCA cycle in this heart was derived from [3-^{13}C]pyruvate and the total anaplerotic flux was 29% of the flux through citrate synthase.

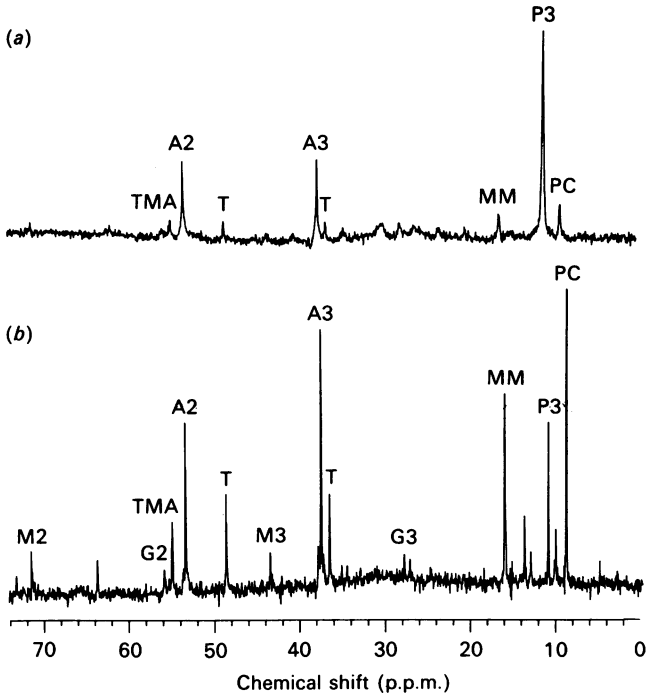

Fig. 4. ^{13}C n.m.r. spectrum of an intact heart (*a*) and an extract of that same heart (*b*) perfused with 2 mM-[3-^{13}C]-propionate for a 2 h period

P3 is the resonance from [3-^{13}C]propionate, most of which is external to the heart. The remaining resonances are labelled as outlined in the legends to Figs. 2 and 3.

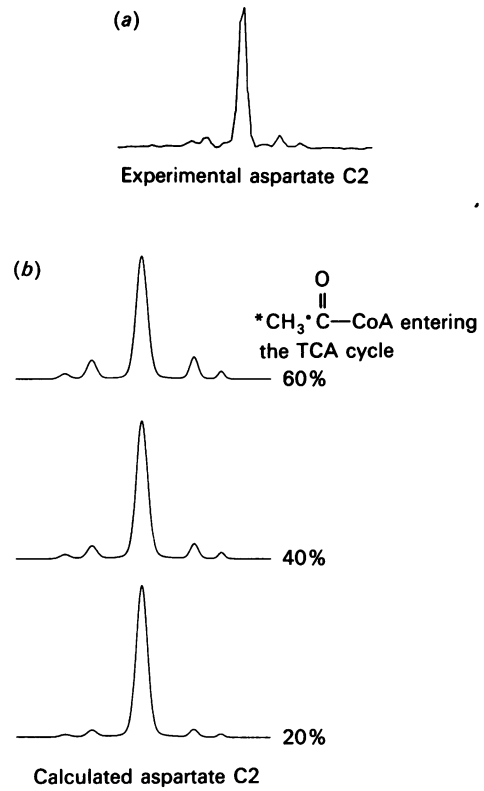

Fig. 5. The expanded aspartate C2 resonance (*a*) from the heart extract spectrum shown in Fig. 4 and calculated aspartate C2 resonances (*b*) for 20%, 40% or 60% ^{13}C fractional enrichment of acetyl-CoA entering the TCA cycle

As indicated, acetyl-CoA derived from [3-^{13}C]propionate via pyruvate would have equal probability of being labelled in the methyl (*) or carbonyl (·) carbons. Thus, 20% total enrichment represents 10% ^{13}C enrichment in the methyl carbon and 10% ^{13}C enrichment in the carbonyl of acetyl-CoA.

Spectral differences when the ^{13}C originates with the anaplerotic substrate

Perhaps a more direct means to follow the flux of propionate through the TCA cycle intermediate pools would be to enrich one or more of the propionate carbons with ^{13}C. The spectrum in Fig. 3(*b*) illustrates a ^{13}C spectrum of an extract of a heart that was perfused with the same concentrations of pyruvate and propionate, but with the ^{13}C label switched from pyruvate to the methyl carbon of propionate. The malate, aspartate and citrate resonances now appear as singlets because [3-^{13}C]propionate can produce only singly labelled [2- or 3-^{13}C]malate, [2- or 3-^{13}C]oxaloacetate (not detected), and consequently, [2- or 3-^{13}C]aspartate. Further turns of these metabolites through the TCA cycle would yield only [2- or 3-^{13}C]glutamate unless some of the pyruvate entering via PDH is also labelled. Since glutamate C-4 is derived solely from C-2 of acetyl-CoA which, in turn, is derived from [3-^{13}C]pyruvate, its absence in this spectrum suggests that relatively little of the pyruvate pool available to PDH became enriched during this perfusion period. However, the question remains, how much of the pyruvate pool would need to be derived from [3^{13}C]-propionate (presumably via [2- or 3-^{13}C]malate or oxaloacetate) before glutamate C-4 and ^{13}C–^{13}C coupling in the remaining metabolites would be detectable? In an effort to examine this question more fully, we have perfused hearts with [3-^{13}C]propionate as the sole exogenous substrate for extended periods of time to maximize the possibility of detecting ^{13}C flux from propionate → malate → pyruvate → acetyl-CoA and subsequently into the TCA cycle. The spectrum shown in Fig. 4 (*a*) was obtained from an intact heart during a 2 h perfusion period with [3-^{13}C]propionate. The spectrum in Fig. 4(*b*) was obtained from an extract of the same heart. Approx. 25–30% of the labelled propionate was consumed by the heart during the 2 h perfusion as judged by its disappearance from the perfusion chamber. This should have contributed a significant fraction of [2- or 3-^{13}C]pyruvate to the available pyruvate pool if indeed the labelled cycle intermediates were disposed of via the malic enzyme (Hiltunen & Davis, 1981). The steady-state malate and glutamate concentrations were too low to detect in the spectrum of the intact heart during perfusion with propionate as the sole exogenous substrate. These resonances are visible in the extract spectrum but the poor signal-to-noise precludes detecting any spin–spin coupling in these resonances. The glutamate C-4 resonance is once again notably absent or at least within the noise level of this spectrum. The aspartate resonances, however, provide a similar history of ^{13}C flux through the TCA cycle as outlined previously for glutamate (Malloy *et al.*, 1988). Fig. 5 illustrates how the aspartate C-2 (or C-3) resonance would change as a function of fractional ^{13}C enrichment of acetyl-CoA entering the TCA cycle and compares these calculated

results with an expanded plot of the aspartate C-2 resonance taken from the extract spectrum of Fig. 4(*b*).

Assignment of methylmalonate and propionylcarnitine resonances in the intact heart

Two ^{13}C resonances, previously unreported in tissue, were always observed in hearts perfused with [3-^{13}C]-propionate [labelled MM and PC in Figs. 3(*b*) and 4]. Resonance MM is readily assigned to [*Me*-^{13}C]-methylmalonate based upon a chemical shift comparison to an unenriched methylmalonate standard at pH 7. [*Me*-^{13}C]Methylmalonate has also been synthesized and the chemical shift of the methyl resonance is reported to be at 16.0 p.p.m. (Robbins *et al.*, 1986). From our previous observation of acetylcarnitine in [2-^{13}C]acetate perfused hearts (Malloy *et al.*, 1987; Sherry *et al.*, 1985) we expected that resonance PC might arise from propionylcarnitine (see Fig. 1). This was verified by enzymatic synthesis of [3-^{13}C]propionylcarnitine by mixing [3-^{13}C]propionate, CoA, ATP, carnitine, acetyl-CoA synthase, and carnitine acyltransferase in a n.m.r. tube. The only resonance observed (spectrum not shown), in addition to the [3-^{13}C]propionate resonance at 11.0 p.p.m., was a new methyl resonance at 9.0 p.p.m. which we assign to [3-^{13}C]propionylcarnitine. A second experiment starting with [1-^{13}C]propionate produced a new carbonyl resonance at 176.7 p.p.m. which we assign to [1-^{13}C]propionylcarnitine. Both samples regenerated propionate (as determined by n.m.r.) after base hydrolysis.

Fig. 6 shows a ^{13}C n.m.r. spectrum obtained from an intact heart perfused with unenriched pyruvate and [1-^{13}C]propionate. This illustrates that the carboxyl carbons of malate and aspartate and the carbonyl of propionylcarnitine become enriched under these conditions. The carboxyl resonance of methylmalonate is degenerate with the malate C-4 resonance, but the greater intensity of malate C-4 over malate C-1 suggests that methylmalonate is indeed present. The glutamate C-1 and fumarate C-1 and C-4 resonances also overlap so their presence or absence cannot be determined with certainty. Although this spectrum does not provide further new information about propionate metabolism in the heart, it does verify the assignment of resonances in the [3-^{13}C]propionate perfused hearts. This spectrum also illustrates that carboxyl-enriched metabolites may be detected in certain situations *in vivo* where proton decoupling may be prohibitive or may compromise the tissue.

Finally, our observation of [*Me*-^{13}C]methylmalonate in perfused hearts raises an interesting question about its possible metabolic function. Montgomery *et al.* (1983) have proposed that the propionate → succinate pathway occurs at least partially via a shunt involving free methylmalonate. We have examined the possible direct utilization of methylmalonate by the heart by perfusing a single heart with 2 mM-sodium [*Me*-^{13}C]methylmalonate as its sole exogenous substrate. The ^{13}C spectrum of the extract of that heart showed only a small methylmalonate resonance at 16 p.p.m., perhaps entirely due to residual perfusate within the heart during freeze-clamping, and predominant resonances from natural abundance taurine (spectrum not shown). This result is consistent with a failure of the heart to remove methylmalonate from the perfusion medium.

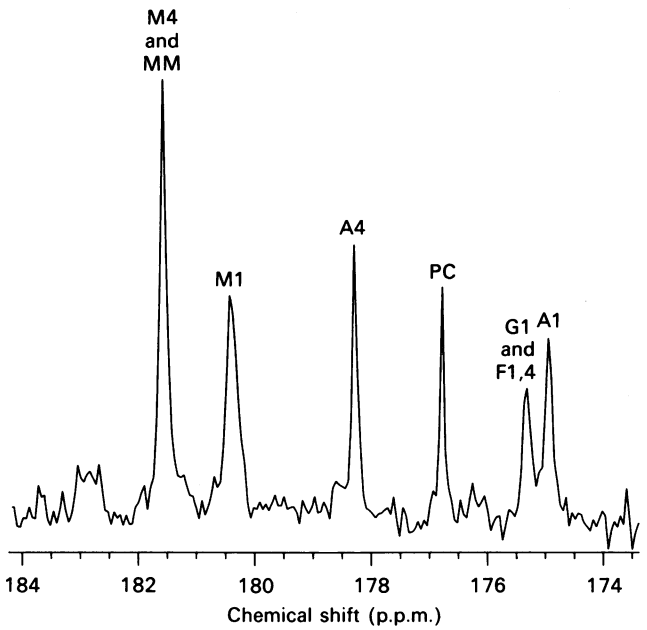

Fig. 6. ^{13}C n.m.r. spectrum (carboxyl region only) of an intact heart perfused with 2.5 mM-pyruvate plus 2 mM-[1-^{13}C]propionate

The resonances are labelled as outlined in the legends to Figs. 2 and 3. F1,4 corresponds to the fumarate carboxyl resonance.

DISCUSSION

We have used ^{13}C n.m.r. to examine the fate of propionate carbons in perfused rat hearts. The spectra are remarkably different in hearts perfused with a combination of pyruvate plus propionate, depending upon which substrate contains the enriched ^{13}C nucleus (Fig. 3). When pyruvate contains the label, those molecules which are formed directly from pyruvate external to the TCA cycle, i.e. alanine via alanine transaminase and lactate via lactate dehydrogenase, become labelled and hence appear in the spectrum. Similarly, when the ^{13}C originates with propionate, two different molecules, methylmalonate and propionylcarnitine, which are formed from intermediates along the propionate pathway (Fig. 1), become labelled and are observed in the spectrum. Alanine and lactate are not detected (though present) when the ^{13}C label originates in propionate and methylmalonate and propionylcarnitine are not detected (though present) when the ^{13}C label originates in pyruvate. The remaining metabolites visible in these spectra are derived from the TCA cycle or related reactions and consequently are present regardless of the origin of the ^{13}C label.

The isotopomer analyses of hearts perfused with [3-^{13}C]pyruvate alone versus a mixture of [3-^{13}C]-pyruvate and unenriched propionate indicate that unenriched propionate does indeed enter the TCA cycle as an anaplerotic substrate (the anaplerotic flux increased from 18% to 29% in pyruvate versus pyruvate plus propionate perfused hearts, respectively) and results in dilution of ^{13}C in the pyruvate pool available to PDH (the ^{13}C fractional enrichment in acetyl-CoA entering the TCA cycle drops from 92% to 65% in pyruvate versus

pyruvate plus propionate perfused hearts, respectively). This would suggest that 27% (92% − 65%) of the pyruvate molecules available to PDH are derived from propionate under our perfusion conditions. This, of course, assumes that other endogenous sources of acetyl-CoA are not stimulated when propionate is added. This analysis does not identify the disposal pathway(s) which convert(s) a TCA intermediate to pyruvate, but we submit that our 27% measurement is less equivocal than corresponding $^{14}CO_2$ measurements (Hiltunen & Davis, 1981; Sundqvist *et al.*, 1984; Latipaa *et al.*, 1985) because it is more direct and also insensitive to possible scrambling of CO_2 via other reactions.

At first glance, the absence of coupling in the spectra of hearts perfused solely with ^{13}C-labelled propionate (Fig. 3*b* and Fig. 4) suggests that very little ^{13}C is leaving the TCA cycle intermediate pools and re-entering the cycle via acetyl-CoA. However, it has been shown previously (Malloy *et al.*, 1988) that multiplet structure is detectable only when the ^{13}C fractional enrichment of acetyl-CoA is above ~ 20% at a single carbon. If the two acetyl carbons were labelled with equal probability, the total acetyl-CoA enrichment could be as high as 40% (total of C1- and C2-enriched molecules) before multiplet structure would be observed above the noise level. During a 2 h perfusion with [3-^{13}C]propionate as the sole exogenous substrate, approx. 120 μmol of [3-^{13}C]propionate was utilized by a heart. This would have produced 120 μmol of [2-^{13}C] or [3-^{13}C]malate and, consequently, 120 μmol of [2-^{13}C] or [3-^{13}C]pyruvate, if disposal occurs via the malic enzyme. If one assumes a maximum TCA cycle flux of 12 μmol/min per g of dry wt. (Sundqvist *et al.*, 1984; Latipaa *et al.*, 1985), this 200 mg (dry wt.) heart would have utilized 288 μmol of acetyl-CoA during this 2 h period. The aspartate C-2 resonance in the n.m.r. spectrum of that heart extract (Fig. 4*b* and expanded in Fig. 5*a*) shows that aspartate C-1 and C-3 are partially enriched (since $J_{12} \neq J_{23}$, the C-2 resonance appears as two doublets asymmetrically positioned about the central singlet). The calculated spectra in Fig. 5(*b*), based upon our model of ^{13}C flux through the TCA cycle (Malloy *et al.*, 1988), illustrate the relative insensitivity of the aspartate spectrum to the amount of label entering via acetyl-CoA. The experimental spectrum indicates that approx. 40% of the acetyl units entering the TCA cycle were labelled in the C-1 or C-2 positions. This corresponds to essentially all of the [3-^{13}C]propionate consumed by the heart during this 2 h perfusion (0.4 × 288 μmol ≅ 120 μmol). Thus, all of the [3-^{13}C]propionate consumed by the heart appears to have entered the oxidative pathway as [2-^{13}C]pyruvate or [3-^{13}C]pyruvate, consistent with disposal of malate via the malic enzyme (Latipaa *et al.*, 1985). The remaining 170 μmol of acetyl-CoA utilized by the heart during this 2 h perfusion period was derived from unknown endogenous sources containing natural abundance ^{13}C.

Our observation of ^{13}C-enriched methylmalonate and propionylcarnitine resonance in intact hearts and in heart extracts suggests that it might be possible to monitor these resonances directly in animals or humans with methylmalonic aciduria, propionic acidaemia, or another abnormality involving the propionate pathway. Although methylmalonate is metabolized when injected intraperitoneally into rats (Montgomery *et al.*, 1983), we find that methylmalonate is not taken up by perfused heart to any significant extent during a 30 min perfusion. Thus, the role of this metabolite in the heart remains uncertain.

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