BJ www.biochemj.org

BJ Classics

Biochem. J. (2012) (Printed in Great Britain) doi:10.1042/BJ20120049

CLASSIC PAPER

A great adventure: from quantitative metabolism to the revelation of Chinese science

Robin HESKETH

Department of Biochemistry, University of Cambridge, Sanger Building, 80 Tennis Court Road, Cambridge CB2 1GA, U.K.

By the end of the 1930s, Frederick Gowland Hopkins had built his Cambridge laboratory into the pre-eminent institute of biochemistry that was a magnet for scientists from all over the world. Even so, it was an exceptional event that saw a young Chinese student leave a homeland at that time relatively isolated from the West and embark on a research career in his department. Within 2 years, she had published a highly influential set of papers on metabolism in the *Biochemical Journal*, a field that some 70 years on has once again become a major focus as its role in cancer is dissected. From the outset, however, she had come under the spell of the legendary polymath Joseph Needham to whom she would dedicate the rest of her life in a partnership that would unveil the astounding history of Chinese science to the world.

Key words: glycolysis, history of biochemistry, Lu Gwei-djen, metabolism, pyruvate.

If the definition of 'classic' papers is that they are still being cited many decades after publication, the set of five by Lu Gwei-djen (鲁桂珍) (Figure 1) on the metabolism of pyruvic acid that appeared in the *Biochemical Journal* in 1939 [1–5] certainly meet the criterion (cited in, e.g., [6]). In purely numerical terms, the first of these papers [1], which defined a new method for determining the amount of pyruvate in blood, has been cited over 200 times and remains among the 50 most highly cited papers published in the *Biochemical Journal* before 1940. Its author, Lu Gwei-djen, was the gifted daughter of an apothecary who, through the fortunate chance of being born in the city of Nanjing, had attended Ginling College, the first university in China to award degrees to women. Founded by a group of American women and flourishing to this day as part of Nanjing Normal University, it must have been an intoxicating stimulus for young and alert minds growing up in turbulent times. Teaching was in English, and Lu studied a broad curriculum that included zoology and biochemistry. She then trained as a chemical pathologist at Peking Union Medical College before moving to Shanghai, teaching at St. John's University and joining the Henry Lester Institute in 1933, where she worked on vitamin B_1 deficiency, among other things, with Benjamin Platt. Through this, Lu became aware of the Cambridge biochemistry school, particularly of Joseph Needham's seminal three-volume *Chemical Embryology* [7] and of the work of his wife, Dorothy, on muscle biochemistry. Encouraged by Platt, she applied to Hopkins as a postgraduate student and he asked Dorothy Needham to look after her. An additional incentive was doubtless her awareness of Needham's eclectic interests, especially his concern for political and social issues, gleaned from reading English newspapers.

The passage of some 75 years has made us so familiar with the concept of metabolic pathways that it is perhaps difficult to comprehend the ferment of the field when Lu joined the Cambridge laboratory in 1937. Given that Scheele had isolated lactic acid in 1780 and by 1835 Berzelius had purified pyruvic acid, it seems scarcely credible that, well into the 20th Century, there held sway the notion that within cells 'protoplasm' represented some kind of 'giant molecule' within which chemical events occurred that were not susceptible to dissection and analysis. Hopkins had been a resolute opponent of this view from the earliest stages of his career and he was convinced of the importance of chemical specificity in cellular reactions. A seminal contribution in this regard came from his

Figure 1 Lu Gwei-djen (鲁桂珍) (1904–1991)

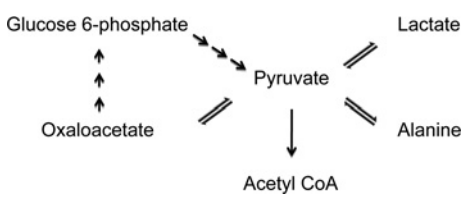

Figure 2 Pyruvate: major metabolic routes

work with Walter Fletcher showing that oxygen depletion causes an accumulation of lactic acid in muscle [8]. The methods they developed established the field of muscle biochemistry and their findings, when combined with the work of Otto Meyerhof and others, led by the end of the 1930s to the delineation of the glycolytic pathway [9] (Figure 2).

By the time the papers by Lu appeared the detail of the glycolytic pathway had therefore largely been revealed and pyruvic acid was established as a normal intermediate in the breakdown of carbohydrate. Even so, it is remarkable that well over 50 methods for its quantitative estimation had by then been published. However, as Lu noted, few were sensitive enough to detect the low level (less than 0.6 mg/100 ml) present in normal blood. The strategy she devised was to convert pyruvic acid into 2,4-dinitrophenylhydrazone, which could then be purified and

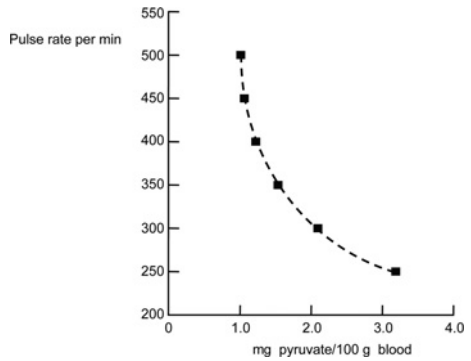

Figure 3 Pulse rate as a function of blood pyruvate levels in rats after 3 weeks on a vitamin B_1-deficient diet

The pyruvate levels were averages of measurements from three to five rats for each point, except for the 450 rate which was a single determination. Redrawn from Figure 1 of [2].

quantified by colorimetry, thereby circumventing the problem of contamination by aldehyde or ketone derivatives [1].

Lu then applied her method to follow changes in blood pyruvate in rats, pigeons, rabbits and humans [2], showing that the level is raised either by vitamin B_1 deficiency or exercise and that as pyruvate increases pulse rate declines (Figure 3).

Figure 3 reproduces the rat data that supported this key finding. In these experiments acute vitamin B_1 deficiency was induced in albino rats by feeding them a devitaminized diet (that included Marmite) for 3 weeks. Lu's careful measurements showed the close correlation between blood pyruvate level and heart rate and that there was a similar association in animals suffering chronic deficiency. In control experiments she demonstrated that injecting pyruvate into the circulation of normal rats and rabbits, raising the blood pyruvate level to many times that found in deficiency states, had no effect on heart rate. Given that subcutaneous injection of vitamin B_1 restored the normal blood pyruvate level within 6 h, Lu concluded that the changes were indeed caused by vitamin B_1 deficiency.

An earlier paper by Platt and Lu [10] had shown that pyruvic acid levels were increased in patients suffering from beri-beri, an observation that was extended by the finding that light exercise raised blood pyruvate in vitamin B_1-deficient humans, with the inference that whole body exercise may reveal latent vitamin B_1-deficiency [3,4]. The linking of abnormal pyruvate metabolism with vitamin B_1 deficiency reflected, of course, the influence of Hopkins. He had been awarded the 1929 Nobel Prize for his work on, primarily, vitamins A and D, but he was a co-recipient with Christiaan Eijkman whose work had led to the identification of thiamine (vitamin B_1) as the deficient entity in beri-beri.

Finally in this series, with Dorothy Needham, Lu investigated the fate of pyruvate *in vivo* by injecting it into rabbits and showing that blood lactate levels rose significantly, leading them to suggest that the reaction was important in the rebuilding of carbohydrate, reflected in the subsequent increase in glucose [5].

By this time Warburg had already reported his finding that cancer cells have increased glucose uptake relative to their normal counterparts, using it for fermentation rather than oxidative phosphorylation, even when oxygen is available [11]. The characteristic of many tumour cells to generate energy through a high glycolytic flux and associated production of lactate thus became the 'Warburg effect', destined to lie disregarded in the archives for the best part of 70 years. Many years later, Warburg also showed that deprivation of vitamin B_1 could switch patterns of cellular metabolism from normal to those he had identified as being associated with cancer cells [12]. More recently, abnormal metabolism has moved into the spotlight of cancer biology, attention having been focused by the potential targets offered both for therapeutic intervention and for tumour detection. We now perceive the Warburg effect as being due to mitochondrial damage or suppression of apoptosis, and as a means of adaptation to hypoxic environments within tumours.

There are four human isoforms of the glycolytic enzyme that produces pyruvate (pyruvate kinase) and, in many tumour cells, PKM2 is the predominantly expressed form. The activity of PKM2 is modulated by oncogenic stress, leading to the diversion of metabolic flux into the pentose phosphate pathway, promoting the Warburg effect. Astonishingly, in human cancer cells, PKM2 executes an additional, quite distinct, function. Signalling from

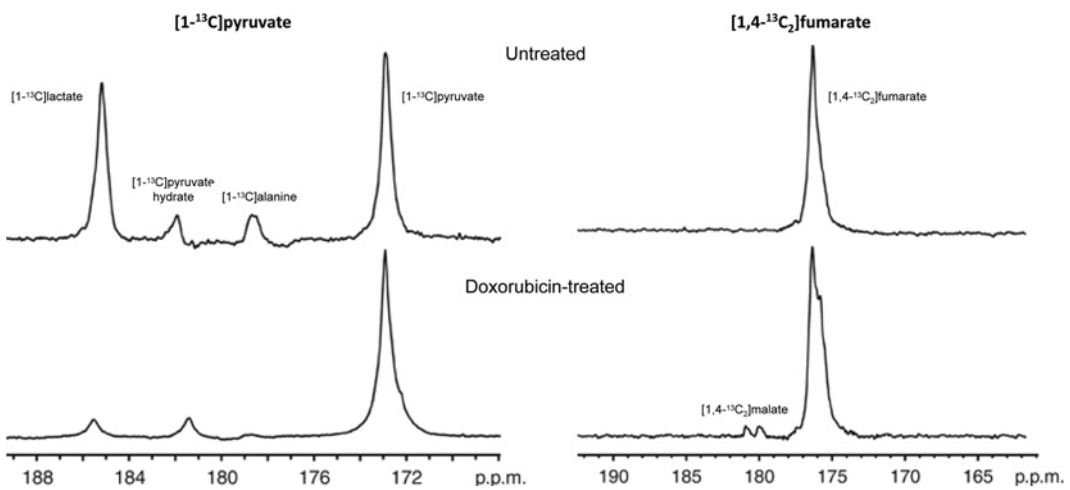

Figure 4 Response of human breast adenocarcinoma xenografts in mice to treatment with doxorubicin (24 h) detected using hyperpolarized [1-^{13}C]pyruvate or [1,4-$^{13}C_2$]fumarate

Pyruvate–lactate exchange decreased by 49 % in this period, whereas the generation of [1,4-$^{13}C_2$]malate from fumarate reflects tumour cell necrosis. Reprinted by permission from Macmillan Publishers Ltd on behalf of Cancer Research UK: British Journal of Cancer {Witney, T.H., Kettunen, M.I., Hu, D.-e., Gallagher, F.A., Bohndiek, S.E., Napolitano, R. and Brindle, K.M. (2010) Detecting treatment response in a model of human breast adenocarcinoma using hyperpolarised [1-^{13}C]pyruvate and [1,4-$^{13}C_2$]fumarate. Br. J. Cancer **103**, 1400–1406}, © 2010; [23].

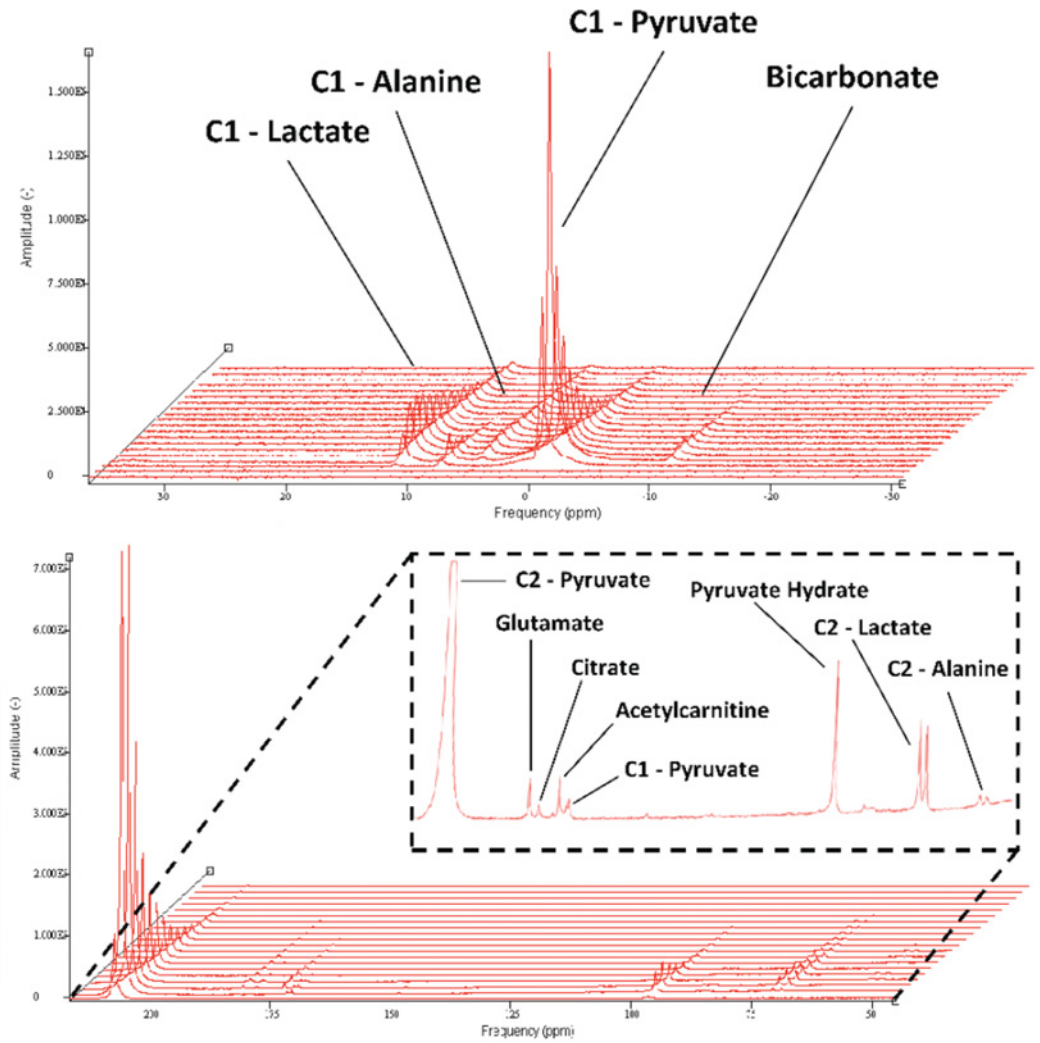

Figure 5 Real-time quantification of metabolism in rat hearts *in vivo*

Upper panel: spectra obtained following injection of hyperpolarized [1-^{13}C]pyruvate: the ratio of bicarbonate to pyruvate resonances provides a measure of the flux through pyruvate dehydrogenase. Lower panel: following injection of hyperpolarized [2-^{13}C]pyruvate, the resonances of several tricarboxylic acid cycle intermediates are visible in real time with a temporal resolution of 1 s. Spectra kindly provided by Dr Damian Tyler, Department of Physiology, Anatomy and Genetics, University of Oxford.

the epidermal growth factor receptor via activated SRC can lead to the transcription of genes that drive cell proliferation [*MYC* and *CCND1* (cyclin D1)], for which β-catenin is an essential transcription factor. However, its association with PKM2 is required for promoter binding. The significance of this role is illustrated by the correlation of nuclear levels of PKM2 with the developmental stage of human glioblastomas and their prognosis [13].

The expansion of the field's horizons illustrated by these results has caused cancer biologists to revisit the subject of metabolite assays, but, in contrast with Lu's time, the emphasis is now on *in vivo* determinations. Approaches have thus far used PET (positron emission tomography) and MRS (magnetic resonance spectroscopy), and tumour metabolites have been detected *in vivo* by both methods. However, a major limitation has been the problem encountered by Lu, i.e. a lack of sensitivity, and this is particularly acute in the context of identifying responses to chemotherapy at an early stage in treatment. The recently introduced method of DNP (dynamic nuclear polarization), in which a hyperpolarized ^{13}C-labelled substrate is injected into the circulation, increases sensitivity over 10000-fold. This phenomenal enhancement is partly offset by the short half-life of the polarized species, which means that images must be obtained within 5 min of injection. Nevertheless, the method shows great promise and, in a mouse model of human breast adencarcinoma, administration of [1-^{13}C]pyruvate and [1,4-$^{13}C_2$]fumarate revealed tumour metabolism responses by 24 h after drug administration (Figure 4). A first human trial of DNP is in progress, focusing on the metabolism of hyperpolarized pyruvate in prostate cancer.

Corresponding developments in the field of metabolomics have seen hyperpolarized [^{13}C]pyruvate used as a metabolic tracer by infusion into isolated perfused hearts and in whole-animal experiments *in vivo* (Figure 5). The quantification of [1-^{13}C]citrate and [5-^{13}C]glutamate has permitted real-time comparison of the flux through the tricarboxylic acid cycle in normal and ischaemic hearts [14].

These breathtaking advances would doubtless have amazed and delighted Lu, who, in a different world, took the intrepid step of leaving Shanghai for Cambridge to work with Joseph and Dorothy Needham, a story that has been told in fascinating detail elsewhere [15]. Altogether, Lu published only eight research papers, the last in 1941 [1–5,10,16,17]. However, five further papers written jointly with Joseph Needham in the period 1963–1988 [18–22]

reflect the fact that she became Needham's muse and essentially devoted the rest of her life to him and to the realization of the incredible series of 24 volumes that comprise *Science and Civilisation in China* to date. She may well have looked back on her work on metabolism, and particularly her great year of 1939, with justifiable pride. However, it is difficult not to believe that her greatest satisfaction came from her collaboration with Needham in the unique enterprise that has revealed the immense achievements of Chinese science to the world at large. For this, Joseph Needham has rightly become revered in China, but it is only appropriate that the brilliant young woman who left her homeland all those years ago to forge a career in biochemistry is also honoured for her contribution, albeit made in ways that could never have entered her imagination on that day, 75 years ago, when she stepped aboard the steamer bound for England.

ACKNOWLEDGEMENTS

I am most grateful to John Moffett of the Needham Research Institute, Cambridge, for sharing his knowledge of Lu Gwei-djen. Photographs courtesy of the Department of Biochemistry, University of Cambridge.

REFERENCES

1 Lu, G. D. (1939) Studies on the metabolism of pyruvic acid in normal and vitamin B_1-deficient states. I. A rapid, specific and sensitive method for the estimation of blood pyruvate. Biochem. J. **33**, 249–254
2 Lu, G. D. (1939) Studies on the metabolism of pyruvic acid in normal and vitamin B_1-deficient states. II. Blood pyruvate levels in the rat, pigeon, rabbit and man. III. The relation of blood pyruvate to cardiac changes. Biochem. J. **33**, 774–786
3 Platt, B. S. and Lu, G. D. (1939) Studies on the metabolism of pyruvic acid in normal and vitamin B_1-deficient states. IV. The accumulation of pyruvic acid and other carbonyl compounds in beri-beri and the effect of vitamin B_1. Biochem. J. **33**, 1525–1537
4 Lu, G. D. and Platt, B. S. (1939) Studies on the metabolism of pyruvic acid in normal and vitamin B_1-deficient states. V. The effect of exercise on blood pyruvate in vitamin B_1 deficiency in man. Biochem. J. **33**, 1538–1543
5 Lu, G. D. and Needham, D. M. (1939) Studies on the metabolism of pyruvic acid in normal and vitamin B_1-deficient states. VI. The fate of injected pyruvate in the normal rabbit. Biochem. J. **33**, 1544–1548
6 de Almeida, L. C., Avilez, I. M., Honorato, C. A., Hori, T. S. F. and Moraes, G. (2011) Growth and metabolic responses of tambaqui (*Colossoma macropomum*) fed different levels of protein and lipid. Aquacult. Nutr. **17**, E253–E262
7 Needham, J. (1931) Chemical Embryology, Macmillan, New York
8 Fletcher, W. M. and Hopkins, F. G. (1917) Croonian Lecture: the respiratory process in muscle and the nature of muscular motion. Proc. R. Soc. London Ser. B **89**, 444–467
9 Meyerhof, O. (1938) The intermediary reactions of fermentation. Nature **141**, 855–858
10 Platt, B. S. and Lu, G. D. (1936) Chemical and clinical findings in beri-beri with special reference to vitamin B_1 deficiency. Q. J. Med. **5**, 355–373
11 Warburg, O., Posener, K. and Negelein, E. (1924) On the metabolism of carcinoma cells. Biochem. Z. **152**, 309–344
12 Warburg, O., Geissler, A. W. and Lorenz, S. (1970) Producing normal metabolism from cancer metabolism by addition of vitamin-B_1. Z. Naturforsch. B **25**, 559
13 Yang, W., Xia, Y., Ji, H., Zheng, Y., Liang, J., Huang, W., Gao, X., Aldape, K. and Lu, Z. (2011) Nuclear PKM2 regulates β-catenin transactivation upon EGFR activation. Nature **478**, 118–122
14 Schroeder, M. A., Atherton, H. J., Ball, D. R., Cole, M. A., Heather, L. C., Griffin, J. L., Clarke, K., Radda, G. K. and Tyler, D. J. (2009) Real-time assessment of Krebs cycle metabolism using hyperpolarized ^{13}C magnetic resonance spectroscopy. FASEB J. **23**, 2529–2538
15 Winchester, S. (2008) Bomb, Book and Compass: Joseph Needham and the Great Secrets of China, Viking London
16 Lu, G. D. and Needham, D. M. (1941) Adenylic deaminase activity in B_1-avitaminosis and in starvation. Biochem. J. **35**, 392–403
17 Needham, D. M. and Lu, G. D. (1938) The specificity of coupled esterification of phosphate in muscle. Biochem. J. **32**, 2040–2048
18 Lu, G. D. and Needham, J. (1963) China and the origin of examinations in medicine. Proc. R. Soc. Med. London **56**, 63–70
19 Needham, J. and Lu, G. D. (1979) Asia in the making of Europe. J. Asian Stud. **38**, 313–315
20 Lu, G. D. and Needham, J. (1979) Scientific basis for acupuncture. Sciences (N.Y.) **19**, 6–10
21 Lu, G. D. and Needham, J. (1988) A history of forensic medicine in China. Med. Hist. **32**, 357–400
22 Lu, G. D., Needham, J. and Phan, C. H. (1988) The oldest representation of a bombard. Technol. Cult. **29**, 594–605
23 Witney, T. H., Kettunen, M. I., Hu, D.-e., Gallagher, F. A., Bohndiek, S. E., Napolitano, R. and Brindle, K. M. (2010) Detecting treatment response in a model of human breast adenocarcinoma using hyperpolarised [1-^{13}C]pyruvate and [1,4-$^{13}C_2$]fumarate. Br. J. Cancer **103**, 1400–1406

Robin Hesketh has been a member of the Department of Biochemistry at the University of Cambridge and a Fellow of Selwyn College for over 30 years. He works mainly on anti-angiogenic strategies for cancer therapy and his popular science book on cancer, *Betrayed by Nature*, was published recently by Macmillan.
email: t.r.hesketh@bioc.cam.ac.uk