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EDITORIAL

THE FUNCTION OF A VITAMIN: THE LEGACY OF THIS CONCEPT TO BIOCHEMISTRY*

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IN 1929, the year when the Nobel Prize for Medicine was awarded jointly to Eijkmann and Hopkins for their work in pioneering the discovery of vitamins, the mechanisms by which these substances acted inside the body and the reasons whereby their absence from our diet resulted in the signs and symptoms of the different deficiency diseases, were utterly unknown. Yet only seven years later, in 1936 in a lecture delivered at the National Hospital for Nervous Diseases, Queen Square, Peters[1] was able to state that vitamin B_1 "is a catalyst needed for the oxidative removal of one of the lower degradation products of carbohydrate metabolism", and he added that "the biochemical lesion is most closely related to the oxidation of pyruvic acid". Thus, for the first time, the action of a vitamin inside the body was defined in terms of a metabolic process mediated normally only in the presence of that vitamin. This was a classic occasion, since for one of the vitamins at any rate we were now able to account for its function in biochemical terms: vitamin B_1 had now been described as an essential co-enzyme required for the oxidative metabolism of pyruvate in the cells of the nervous system.

The experimental work carried out by Peters and his colleagues during these years has been fully reported in a series of papers in the *Biochemical Journal* and elsewhere, and there is no need to describe it in any detail here. Suffice it to say that a remarkable output of new facts was obtained in a surprisingly short period of time, using a colony of rice-fed pigeons and a series of Barcroft microrespirometers, as a result of which it was possible for Peters to reach this conclusion as to the intracellular function of this vitamin.

It is important also to realise that, in addition to the *in vitro* evidence obtained with the avitaminous brain, *in vivo* evidence of abnormal pyruvate metabolism in the thiamine-deficient pigeon was obtained at an early stage in this work. Experiments carried out in 1933 and 1934 showed that there was an accumulation of pyruvate in the blood of avitaminous pigeons, and also that these high blood pyruvate levels could be rapidly returned to within the normal range by the administration of vitamin B_1

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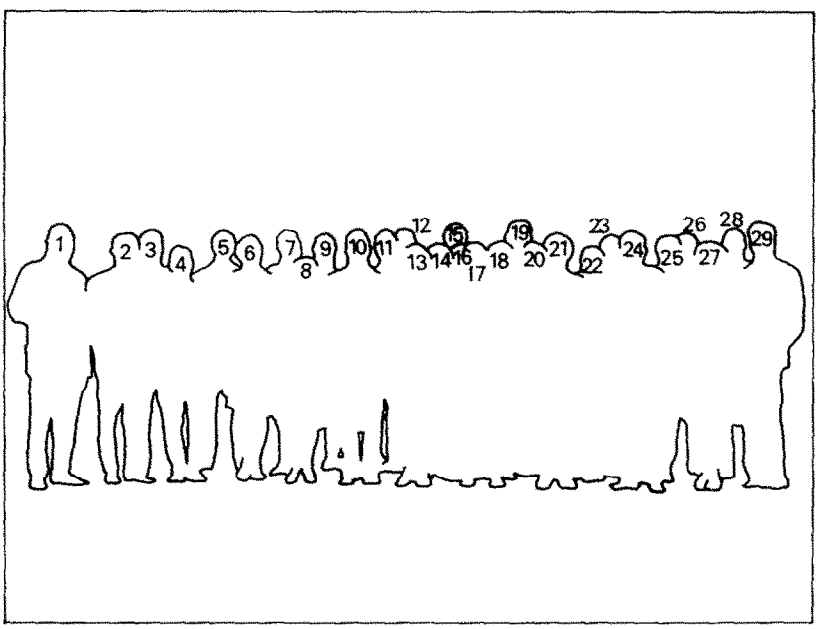
Participants of the Symposium on "Some Aspects of Biochemical Pharmacology" held in honour of Sir Rudolph Peters on June 20th, 1970 at Headington Hill Hall (photograph taken outside Lady Margaret Hall).



(1) V. P. Whittaker, (2) C. Liebecq, (3) P. Buffa, (4) H. McIlwain, (5) J. St. L. Philpot, (6) M. G. Ord, (7) P. Alexander, (8) Lady Peters, (9) W. N. Aldridge, (10) Sir R. A. Peters, (11) R. H. S. Thompson, (12) D. B. Lindsay, (13) A. Beloff Chain, (14) G. H. Lathe, (15) J. M. Barnes, (16) D. S. Parsons, (17) J. R. P. O'Brien, (18) J. D. Judah, (19) H. M. Sinclair, (20) A. E. M. McLean, (21) R. B. Fisher, (22) Sir H. A. Krebs, (23) M. Welsch, (24) Z. M. Bacq, (25) D. W. Kent, (26) G. F. Richards, (27) M. Shorthouse, (28) L. A. Stocken, (29) A. S. Lowe.

to the deficient birds.[2] And very shortly after this the late Professor Platt and Miss Lu,[3] working in Shanghai, were able to show that there is also an accumulation of pyruvate in the blood of patients with beri-beri.

These observations therefore opened up the possibility that the estimation of the blood pyruvate level might provide a useful diagnostic indication of thiamine deficiency. But it soon became clear that the relatively minor degrees of thiamine deficiency that are likely to be seen clinically in this country or in the U.S.A. are not usually associated with any obvious elevation of the fasting blood pyruvate level. To reveal these less severe degrees of thiamine deficiency it is necessary to give a loading dose of glucose—that is to say, to subject the tissues to an abnormal level of pyruvate formed intracellularly from the absorbed glucose. This was first demonstrated by Elsom *et al.*[4] in 1940 in induced thiamine deficiency, and by Bueding, Stein and Wortis[5] in the following year in Wernicke's syndrome and in peripheral neuropathy associated with chronic alcoholism. These findings were soon confirmed by many workers, and the "pyruvate tolerance test" is now an accepted method for the investigation of suspected thiamine deficiency.

It also demonstrates the value of estimating not merely the level of a vitamin in blood or urine, which may give us little more information than indicating the degree of saturation with respect to that vitamin, but rather the level of a metabolite which accumulates intracellularly in the first instance in the absence of the vitamin, and which provides us therefore with an index of the severity of the intracellular biochemical lesion resulting from the deficiency of the vitamin.

This concept has since been taken up and used in other instances, such as the FIGLU test for folate deficiency, or the estimation of the urinary excretion of methyl malonic acid in vitamin B_{12} deficiency.

It is of course now clear, when we take into account our present knowledge of the complexity of the system of enzymes and co-enzymes which are responsible for the oxidative decarboxylation of pyruvate, that a thiamine deficiency is only one of a number of ways in which this reaction could be inhibited, and that there are therefore a number of different causes of elevated blood pyruvate levels. As with most tests used in clinical biochemistry considerable caution has therefore to be exercised in interpreting the significance of raised pyruvate levels.

An interesting recent extension of this work, for example, has been the confirmation by Buckle[6] of the earlier finding that the blood pyruvate level is also increased in untreated cases of subacute combined degeneration of the cord,[7] and that treatment limited to parenteral administration of vitamin B_{12} causes a rapid return to normal of these levels. But Buckle has also shown that in addition to the abnormal elevation of pyruvate, the level of α-oxoglutarate is abnormally low in these patients, a finding which is of interest when it is recalled that a cobamide co-enzyme is required for the conversion of propionic to succinic acid via methyl malonyl co-enzyme A.[8]

A further metabolic function of thiamine pyrophosphate was discovered in 1953, when Horecker and Smyrniotis[9] and Racker, de la Haba and Leder[10] independently described experiments indicating that it was also required as a co-enzyme of transketolase. This demonstration that the pentose phosphate pathway is also thiamine-dependent is of considerable interest in connection with the overall metabolic abnormalities that are present in thiamine-deficiency. Dreyfus,[11,12] among others, has shown that the level of erythrocyte transketolase undergoes a progressive reduction

during the development of a thiamine deficiency. At a Ciba Foundation Study Group which met in 1967 this problem was discussed at some length, and it was clear that although striking abnormalities of transketolase activity certainly occur in Wernicke's encephalopathy, much more work is needed to evaluate the usefulness of this estimation in milder degrees of thiamine deficiency.

In the years that followed the discovery that thiamine pyrophosphate functions as a co-enzyme, this concept was of course applied to the other members of the water soluble B group of vitamins, and their functions as co-enzymes in the various systems responsible for electron and proton transport, for amino acid decarboxylations, for transamination, for carboxylation and for the transport of acetyl groups and of 1-carbon units is now common knowledge. But although large areas of biochemistry have been considerably elucidated by this work, there are further developments that are being examined at the present time. I am thinking here, for example, of the evidence that is now accumulating linking vitamin B_{12} with cyanide metabolism—evidence that is being obtained by Wilson *et al.* not only in this country, but also in those areas of the tropics where cassava is a staple article of diet, and where peripheral neuropathies, due almost certainly to the chronic cyanide ingestion derived from the cassava, are a common and severely disabling problem.

Mention of the toxicity of cassava leads on to a further area of biochemical and toxicological development that can fairly be said to have emerged very largely from Peters' early work on thiamine.

Shortly before the outbreak of World War II Peters returned to the problems of chemical defence. His interest in this field of toxicology goes back indeed to World War I when, as he has said in one of his writings, the first gas attacks gave him an emotional push from which he has never quite recovered. In 1939 therefore he set up a group in the Oxford laboratory to work on those problems which at that time seemed of most immediate military significance. One of these problems was to analyse the mode of action of lewisite (chlorovinyldichloroarsine), and if possible to produce an antidote to it.

Following on his thiamine work he had reported in 1936 that the vesicant substance dichlorodiethylsulphone had a selective action on the oxidation of pyruvate by brain,[1,13] and in the following year he found that sodium arsenite had a similar effect. It had indeed been known since 1911, from work carried out by Onaka, that sodium arsenite inhibited the respiration of nucleated red blood cells, and Krebs[14] in 1933 had used it to inhibit α-keto acid oxidation in kidney. The idea emerged therefore that inactivation of an enzyme system might be directly responsible for the pathological effects produced by vesicant substances, and, using the pigeon brain, it was shown that, of a number of enzymes studied, the pyruvate oxidase system proved to be outstandingly the most sensitive both to sodium arsenite and to lewisite.

The next step, to find the chemical basis of this high degree of sensitivity of the pyruvate oxidase system to poisoning by arsenicals, also takes one back to some earlier work carried out by Peters. As early as 1909 Ehrlich had suggested that the chemoreceptors for arsenic might be hydroxyl or thiol groups, and in 1924 Walker and Peters had obtained experimental evidence definitely connecting the action of another arsenical, diphenylchloroarsine, which had been of interest as a chemical agent in World War I, with thiol groups.

And in the intervening years between the two wars the work of Voegtlin and his

colleagues in America, and of others in this country and elsewhere, produced further evidence that arsenic exerts its toxic effects by reaction with tissue thiols.

However, the addition of even large excesses of simple monothiol compounds such as cysteine proved quite ineffective in protecting the pyruvate oxidase system from the toxic action of lewisite.

Experiments with a model thiol-containing protein, kerateine, however, yielded results suggesting that the high toxicity of trivalent arsenicals might be due to their ability to combine with two closely situated SH groups of some component of the pyruvate oxidase system, and that efficient protection against these arsenicals would only be afforded by the presence of a competing dithiol.[15] And so 2:3-dimercapto-propanol (British Anti-Lewisite, or dimercaprol), was prepared, tested and found to be an effective antidote.[16]

Although the complications of arsenical therapy are fast disappearing from the clinical scene, dimercaprol still remains as a useful treatment for acute arsenical poisoning, and has also been used for the mobilization and removal of copper in Wilson's disease.

It is also interesting, in retrospect, to recall the work of Reed *et al.*,[17] which led to the discovery of the participation of lipoic acid in the oxidative decarboxylation of pyruvate. In 1947, at the second of the Biochemical Society Symposia, devoted to "The Biochemical Reactions of Chemical Warfare Agents", it was possible to conclude,[18] on the basis of the lewisite work, that the pyruvate oxidase system probably includes a dithiol component, while other thiol-containing enzymes, less sensitive to arsenic, and capable of being protected by monothiols as well as by dithiols, could be regarded as "monothiol enzymes". Six years later the postulated dithiol component was isolated from beef liver by Reed and his colleagues,[17] and was indentified as lipoic acid, 6,8-dithiooctanoic acid, to be followed shortly by the full elucidation of the part played by it in the formation of acetyl-co-enzyme A from the thiamine pyrophosphate acetaldehyde intermediate.

Another of the many other aspects of Peters' interest in toxicological problems must be referred to briefly, since, again, it has led to the establishment of a principle in biochemistry, and this is his more recent work on fluoroacetate, the toxic component of the S. African plant *Dichapetalum cymosum*. This work, again, is now well known, and there is no need to describe it in any detail here. But apart from the wider toxicological implications, it is of biochemical importance on two counts. In the first place Buffa and he[19] were able to show that in fluoroacetate-poisoned animals citrate accumulates and can be detected in large amounts in the brain. They thus provided *in vivo* evidence that the citric acid cycle is part of the system responsible for the oxidation of carbohydrate by cerebral tissue, that is to say, they showed that Sir Hans Krebs' earlier *in vitro* work and conclusions on the tricarboxylic acid cycle applied equally to the intact animal. And secondly, he showed that it was the synthesis of fluorocitrate from fluoroacetate which was responsible for blocking the cycle at the aconitate hydratase stage, a finding which led him to put forward the important concept of what he has called "lethal syntheses" taking place inside the body.[20] Further examples of this are already known, and one can confidently expect that such lethal conversions will account for the mechanism of toxicity of many other substances, whose mode of action is at present unknown.

These are only selected items from his extensive repertoire. Nothing has been said

about his earliest work of all, begun in 1911, on the specific oxygen capacity of haemoglobin, or about his long-lasting interest in cell surfaces and in the organization of cellular activities that led him in 1929 to put forward his concept of the "cytoskeleton"[21] a concept that has since blossomed into the now prolific study of intracellular organelles and their functions and interrelations.

Although much of what has been mentioned above is now common knowledge among biochemists, the purpose of this paper has been to attempt to trace some of the biochemical thinking and researches of Sir Rudolph Peters and to link his concept of the biochemical lesion which emerged from his early work on thiamine deficiency with some of the more recent advances in biochemistry, pharmacology and pathology.

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