Regulation of Cell Metabolism

Society Colloquium held in honour of the 80th birthday of Sir Hans Krebs, F.R.S., organized by M. W. Fowler (Sheffield) and D. H. Williamson (Oxford) and edited by D. H. Williamson (Oxford)

Tribute to Sir Hans Krebs

Sir Hans Krebs was eighty on 25 August 1980. To mark this event the Biochemical Society held a Colloquium in Sheffield on 23 July 1980 organized by M. W. Fowler (University of Sheffield) and D. H. Williamson (Oxford). Many former colleagues and students of Sir Hans attended the Colloquium and the reunion party in his honour on the previous evening. The general theme of the Colloquium was metabolic regulation, and the specific topics dealt with included: urea synthesis, purine metabolism, gluconeogenesis and ketogenesis; all areas in which Sir Hans has made outstanding contributions. The speakers at the Colloquium were: H. L. Kornberg, J. R. Williamson, R. W. E. Watts, E. A. Newsholme, H.-D. Soling, P. J. Randle, K. G. M. M. Alberti, M. Koike and S. Numa (the last two contributors were nominated by the Japanese Biochemical Society).

Hans Krebs was born in 1900 in Hildesheim, a small town in northern Germany. His father was an ear, nose and throat surgeon and played a major role in his son's decision to take up medicine. A second key factor in shaping the subsequent development of Sir Hans's research interests was his appointment as research assistant in the laboratory of Otto Warburg. This was a golden age of German biochemistry, and the Kaiser Wilhelm Institute, Berlin, was one of the centres of excellence. It was from Warburg that he learnt the simple but elegant techniques of manometry, spectrophotometry and preparation of tissue slices that were to form the basis for much of his future research.

After five fruitful years with Warburg, Sir Hans was appointed Assistant in Medicine in the University of Freiburg. It was here that he and a clinical student, K. Henseleit, demonstrated that liver slices readily synthesized urea from ammonia and sources of carbon and energy, and that ornithine and citrulline acted catalytically in this process. The description of the first metabolic cycle (1932) was rapidly recognized as a major achievement by the international biochemical community. Shortly after publication of this work Sir Hans was dismissed from his post in Freiburg under the anti-Semitic legislation introduced by Hitler. He emigrated to England (1933) and accepted Sir Frederick Gowland Hopkins's invitation to join the Department of Biochemistry in Cambridge.

In Cambridge, he continued the work on amino acid metabolism that he had commenced in Freiburg. He demonstrated the formation of glutamine from glutamate and ammonia and its hydrolysis in animal tissues. This was followed by studies on the synthesis of uric acid by avian liver and on the formation of ketone bodies (with the late N. L. Edson). These two areas of metabolism continued to occupy his interest over the years.

In 1935, Sir Hans was appointed Lecturer in Pharmacology at Sheffield, and this was converted into a Lectureship in Biochemistry three years later. It was during this early period in Sheffield that Sir Hans and a Ph.D. student, W. A. Johnson, performed the experimental work (mainly on pigeon breast muscle) that resulted in the formulation of the tricarboxylic acid cycle or 'Krebs cycle' for the complete oxidation of substrates. This outstanding piece of research is today one of the cornerstones of metabolic biochemistry.

In 1945 Sir Hans was appointed the first Professor of Biochemistry of the University of Sheffield and simultaneously Honorary Director of the newly established Medical Research Council Unit for Research in Cell Metabolism. The Department and Unit expanded rapidly and gained an international reputation. Many former members of the Unit are now University Professors.

On the retirement of Sir Rudolph Peters from the Whitley Chair of Biochemistry in Oxford, Sir Hans was appointed to this position and the Unit moved with him. His main interests during this period were in the developing area of metabolic regulation,
Adenylate cyclase activity and glucose transport in *Escherichia coli*

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The growth of *Escherichia coli* on substrates of the phosphoenolpyruvate-dependent phosphotransferase (PT) system is strongly inhibited by non-catabolizable analogues of glucose, such as 5-thio-D-glucose (Kornberg & Watts, 1978). Mutants that are resistant to this glucose analogue but that are only slightly impaired in their ability to grow on glucose are altered in a gene *tgS*, located at min 52 on the *E. coli* linkage map (Bachmann et al., 1976) and co-transducible with the *cysA* and *ptsH* markers; the gene order is probably ... *cysS tgs tpsL pts H* ... (Kornberg & Watts, 1979). There are two characteristic features of *tgS* mutants. (i) Glucose is utilized simultaneously and to the same extent as other sugars that may be present in the medium. In *tgS* strains, glucose virtually excludes other sugars (Amaral & Kornberg, 1975). (ii) The adenylate cyclase activity of cells rendered permeable by treatment with toluene (Salomon et al., 1974; Harwood & Peterkofsky, 1975) is considerably lower than that of similarly treated *tgS* cells.

We now report experiments that show that these two dysfunctions can be independently suppressed: they are therefore not causally related.

(i) *Restoration of preference for glucose without augmentation of adenylate cyclase activity*

Strains of *E. coli* that carry the *mgl* and *galP* markers and that lack the active-transport systems for galactose can still grow on that hexose. In such strains, galactose is taken up by facilitated diffusion, mediated by the principal Enzyme II for glucose transport, enzyme PtsG (Kornberg & Riordan, 1976). Mutants that are *ptsG* grow very poorly on agar plates containing galactose as carbon source; if, in addition, they are *tgS*, no growth is observed over at least 2 days. In strain PW 26, the *ptsI* gene had additionally been replaced by *ptsP*; the Enzyme I of the phosphotransferase system in this strain was active at 30°C but inactive at 37°C and above. Mutants of PW 26 were selected for their ability to grow on galactose at 30°C. Among them were some that did not grow on this hexose at 37°C; they were still *ptsP* *mgl galP* but contained an alteration, designated *ptsG*, that was co-transducible with *ptsG* and that restored the sensitivity to 5-thio-D-glucose and the utilization of glucose in preference to sugars taken up via the phosphotransferase system. One such mutant, PW 31, was studied further: it was shown still to carry the *tgS* marker, since that could be transferred by transduction into other strains, and its adenylate cyclase activity was as low as observed with the parent *tgS* strain.

(ii) *Alterations of adenylate cyclase activity without restoration of the preferential utilization of glucose*

Two types of *tgS* mutants were obtained that had adenylate cyclase activities at least as high as those normally observed in *tgS* strains of *E. coli* but that exhibited the loss of preference for glucose characteristic of *tgS* strains.

(a) One type carried a gene, designated *cyaS*, co-transducible with the structural genes for adenylate cyclase, *cya*, and for the utilization of *β*-glucosides, *bgl*, at min 83 on the *E. coli* linkage map. Strain PW 193 (*ptsI* *tgS cya bgl*) has negligible adenylate cyclase activity and, in consequence, both the *cya* and *tgS* mutations, does not grow on fumarate unless cyclic AMP is also supplied. When infected with phage P1 that had been propagated on *bgl cyaS*-donor strains, all the transductants selected on fumarate were *Bgl* and had high adenylate cyclase activity, but were still able to carry the *tgS* marker. They were resistant to 5-thio-D-glucose, and utilized glucose and fructose simultaneously and to the same extent.

(b) The second type of mutant carried a gene, designated *cyAM*, located at min 25 on the *E. coli* linkage map, co-transducible with *purB* but less close to *ptsG* than is the *PtsG* marker. Like *cyA* mutants, *cyAM* strains grow on fumarate even though they either lack Enzyme I activity or carry the *tgS* marker; they are high in adenylate cyclase activity. These strains also utilize glucose and fructose equally. Whereas the *cyaS* mutation was not found to produce any phenotypic effect in an otherwise wild-type strain, the *cyAM* mutation increased the adenylate cyclase activity of otherwise isogenic *cyAM* strains approx. 4-fold.

It has been established (Jones-Mortimer et al., 1977; Kornberg & Watts, 1978) that the *tgS* mutation affects a component of glucose uptake that interacts specifically with the PtsG enzyme system. It appears that, in *cyAM* strains, this component plays a role in ensuring that adenylate cyclase is active. If, as Peterkofsky (1977) suggested, this is brought about by the transfer of phosphate from phosphoenolpyruvate to the membrane-bound adenylate cyclase, the component common both to this process and to glucose uptake may well be the glucose-specific factor III described by Kundig (1974). As a working hypothesis, we postulate that if the function of this component might be as illustrated in Fig. 1, in which its ability to transfer the phosphonyl group to either adenylate cyclase or glucose can be separately modified. Phosphorylation of adenylate cyclase would imply also the existence of a phosphoestase activity (designated *acp* in Fig. 1) that returns adenylate cyclase to the inactive state; loss of this enzyme would be expected to increase the activity of adenylate cyclase irrespective of its state of activation by the phosphotransferase system, which is the observed phenotype of the *cyAM* mutants. Although...