

University of California
Ernest O. Lawrence
Radiation Laboratory

TWO-WEEK LOAN COPY

*This is a Library Circulating Copy
which may be borrowed for two weeks.
For a personal retention copy, call
Tech. Info. Division, Ext. 5545*

METABOLIC COMPARTMENTALIZATION

Berkeley, California

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

To be put in a German Review
book

UCRL-11611

UNIVERSITY OF CALIFORNIA

Lawrence Radiation Laboratory
Berkeley, California

AEC Contract No. W-7405-eng-48

METABOLIC COMPARTMENTALIZATION

V. Moses

August 1964

METABOLIC COMPARTMENTALIZATION

V. Moses

Laboratory of Chemical Biodynamics, University of California, Berkeley*

A glance at the metabolic maps which more and more are adorning laboratories of biochemistry will reveal a highly integrated and apparently exquisitely organized network of biochemical reactions, some catabolic and many synthetic. While the evidence on which these reactions are based is derived from a wide variety of organisms, it is probably true that most of the pathways can and do occur in each cell of each species of living thing, all the way from bacteria to men. One is immediately tempted to wonder how the cell chooses between all these myriad opportunities for metabolic activity, how it arranges that the most appropriate reactions are made use of at the right time, and how the many enzymes and cofactors necessary to catalyze all these activities may be distributed among the various zones and regions of the cellular architecture.

Attempts have been made in recent years to account for the control of some fairly restricted areas of metabolism in terms of enzyme and substrate concentrations, reaction rates, and the availability of cofactors. Two very recent examples of this approach have been applied to a study of glycolysis in ascites tumour cells (12,22). Another type of control mechanism applicable to some biosynthetic mechanisms involves retro-inhibition; that is, inhibition of an enzyme catalyzing an early step in a synthetic pathway by the ultimate

* The preparation of this paper was sponsored by the U.S. Atomic Energy Commission.

product produced (18,25,35). Control mechanisms of these sorts appear to be well substantiated; they seem to apply, however, to reactions which take place all within one phase or limited area of the cell, at least insofar as the intracellular localization of the appropriate enzymes may be deduced from their behaviour when the cell is disrupted. For example, the enzymes of glycolysis are found in the "soluble cytoplasm" of the cells and are mostly not associated with particulate material separable by differential centrifugation.

In some instances, particular enzymic activities are associated specifically with one type of particulate fraction. Campbell, Hogg and Strasine (5) fractionated cells of Pseudomonas aeruginosa and found that hexokinase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, gluconic dehydrogenase, malic dehydrogenase, fumarase, isocitric dehydrogenase, isocitritase and catalase were present only in the soluble cytoplasm. Glucose oxidase and succinic dehydrogenase, on the other hand, were found in a "ghost" fraction, while yet other enzymes (NAD oxidase, adenylic kinase, ATPase, etc.) were obtained from more than one fraction. In other organisms, as we shall see later, many enzyme activities may be detected in more than one subcellular fraction, though the ratios between various enzyme activities may be quite different in the different fractions.

We may thus begin to consider the total map of metabolic activity from two points of view: either all the reactions take place within the cell in such a way that no barriers to diffusion of small molecules exist between the enzymes of one pathway and those of another, or we may suppose such diffusion barriers to exist and the movement of small molecules inside the cell is restricted within definite boundaries. In other words, the metabolic activities of the cell might be physically located within defined and restricted localities, or "compartments", in such a way as to prevent the free movement

of material to all parts of the cell. Considering any particular sequence of reactions, it may be that the pathway occurs entirely within a compartment of a particular type so that the products of the pathway have a limited possibility for further reaction. Alternatively, the same chemical sequence of reactions may take place in more than one intracellular compartment, in correspondingly different environments, so that the product of the sequence located in one compartment may react in quite a different way from the product of the sequence in another, solely as a result of different environmental factors. We might envisage, for instance, the same series of reactions producing the same α -keto acid in each of two or three separate compartments. One compartment might also contain an appropriate enzyme for the reduction of this keto acid, together with a suitable source of reducing power; this would result in the formation of the corresponding α -hydroxy acid. The second compartment might be devoid of the reductase, but possess a transaminase and an amino donor, so that instead of being reduced to an α -hydroxy acid, the α -keto acid is transaminated to an α -amino acid. In the third compartment the fate of the keto acid might be decarboxylation; perhaps two of these activities might be present in the same compartment.

Thus, when we see a junction on a metabolic map we must remember that the map represents the sum of all the cell's metabolic possibilities, and that the junction may exist only in that overall sense, the different routes diverging from the junction not really being derived from a precursor which is physically as well as chemically common to all the subsequent routes.

Such a system of separated reservoirs of metabolic pools might apply equally to the metabolism of compounds presented externally to the cell and to those arising endogenously. To say that a particular type of cell may metabolize a compound (A) presented to it to products B, C, D, and E may be a description of the overall capacity of the cell to convert A to a

number of specified products. Organizationally, the cell may convert A to B at one type of intracellular location, to C at another, and perhaps to some characteristic ratio of D and E at a third. The cell may even be able to metabolize A to F, but only if A is present at the correct locus. Supplying A to the outside of the cell may not ensure its presence at the site where F is synthesized, so that A added to the medium surrounding the cell does not result in the formation of F. However, A, arising internally might indeed be brought in contact with the appropriate enzyme and thus be converted to F.

It is often difficult to study organizational possibilities of this sort since there may be no way of distinguishing between the products of one compartment and those of another. For many years now, workers have sometimes been forced to a conclusion involving compartmentalization to account for their observations, while being unable to offer formal proof that this interpretation is valid. Thus Dormer and Street (9), from a study of the growth of tomato roots supplied with glucose, fructose or sucrose, proposed that sucrose is phosphorylated at the cell surface, and the resultant sugar phosphates metabolized there without gaining access to the cell interior. Free glucose is metabolized inside the cell through many of the same intermediates as those on the pathway of sucrose metabolism. The cell surface may itself be considered a compartment if metabolic activity takes place there without equilibration of material with the inside of the cell. The boundary to this compartment would then be the barrier between the surface and the inside, and there may be no barrier between the surface and the medium.

More recently Rothstein et al. (36) studied the relationship of fermentation to cell structures in yeasts. They lyophilized, and extracted, yeast

cells with acetone to produce a non-viable preparation with respiratory and fermentative capacities. This preparation would ferment glucose, fructose and mannose to carbon dioxide and ethanol, but while hexose phosphates, triose phosphate, pyruvate and endogenous reserves were not fermented, glucose, hexose diphosphate, galactose, pyruvate and endogenous reserves were respired. Rothstein et al. concluded that the enzymes of fermentation exist within the cell in an organized structure distinct from mitochondria. Glucose was able to enter the structures concerned with both fermentation and respiration, while compounds like hexose diphosphate could enter the respiratory structure only and were consequently not fermented. The carbohydrate metabolism of the organism was therefore divided into at least two compartments.

In contrast to the findings with tomato roots (9), invertase in yeast is present mostly in the cell wall (43,44), but is also found at another location within the cell: maltase is present only at an intracellular site (43). Externally supplied sucrose may readily gain access to the invertase in the cell wall, but not to the intracellular enzyme, while maltose is brought into contact with the intracellular maltase by the mediation of an inducible permease system in the cell membrane. Conway and Downey (6) have also studied metabolic compartments in yeast and were able to distinguish at least two chambers on the basis of the abilities of certain compounds to enter the cell from the medium. There were found to exist three classes of substances: those not entering the cells appreciably (inulin and sodium), those entering an outer region rapidly but not penetrating appreciably into the centre (succinate, glycerate, arabinose and glucose) and those diffusing rapidly throughout the whole cell (acetate and propionate). The outer region, it was felt, represented a real

space other than a membrane surface, possibly coincident with the cell wall. Early work by Stier and Stannard (42) suggested that the normal endogenous respiration of baker's yeast was exhibited only by the intact cell and was abolished when the normal compartmental architecture of the yeast is destroyed.

Examples of evidence supporting the concept of compartmentalization appear over the whole field of biochemistry and have often arisen as a consequence of kinetic experiments using radioactive tracers. In the field of photosynthesis, Moses et al. (28) studied the relationship between the metabolic pools of the respiratory and photosynthetic intermediates. The actual compounds in the respiratory and photosynthetic carbon reduction pathways are almost identical, although the two pathways are headed in opposite directions. In the green alga Chlorella it was known that the turnover of material in the photosynthetic carbon cycle is some twenty times faster than that of the respiratory intermediates, and the question arose as to whether the very same chemical entities were involved in photosynthesis (reducing carbon dioxide to sugar) and in respiration (oxidizing sugar to carbon dioxide). By investigating kinetically the distribution of radiocarbon from respired glucose and from photosynthetically fixed carbon dioxide it was shown that the same material does not flow in each pathway, but that the two series of reactions are confined to separate compartments with only a very slow interchange of material between them. Support for this conclusion came from an investigation of the appearance of the products of photosynthesis outside the chloroplasts (19). When whole spinach chloroplasts were exposed to labelled carbon dioxide in the light, compounds of the carbon reduction cycle within the chloroplasts were labelled within a short period of time.

Removal of the chloroplasts from

the reaction mixture by filtration showed that only phosphoglycolate, glycolate and serine were present outside the chloroplasts; all the sugar phosphates remained inside. Glyoxylate, but not glycolate, could enter the chloroplasts when added to the medium, suggesting a glycolate-glyoxylate shuttle to carry "reducing power" produced during photosynthesis from the chloroplast to the cytoplasm. Heber and Willenbrink (15) similarly found that the compounds of the carbon reduction cycle were at first confined to the chloroplasts, though later on some of them, notably those also acting as intermediates in respiration, appeared outside. Other compounds (glucose diphosphate, uridinediphosphoglucose, sucrose, malate and citrate) not involved strictly in the photosynthetic reduction of carbon dioxide, were always found outside the chloroplasts.

The question of compartment separation in the products of photosynthesis has been considered by Smith, Bassham and Kirk (40) with particular reference to amino acid synthesis. Using radioactive carbon dioxide, and measuring the specific radioactivities of amino acids extracted from Chlorella after a period of photosynthesis, it was found that the active pools concerned with the rapid metabolism of alanine, serine, glutamic acid and aspartic acid were each less than half of the total reservoirs of these substances: there must therefore be at least two pools of each of these amino acids. It was shown kinetically that the active pools are formed directly from the carbon reduction and may consequently lie within the chloroplast. The less active pools may be cytoplasmic. Results obtained with labelled acetate indicate that glutamic acid labelled from this precursor is probably different from the glutamic acid labelled from radioactive carbon dioxide in photosynthesis. The glutamate incorporating radiocarbon from acetate may thus be in the postulated cytoplasmic reservoir.

The compartmentalization of amino acid metabolism has been studied in a number of tissues other than photosynthetic organisms. Working with whole animals, Schapira et al. (37) injected labelled glycine into rabbits and examined the specific radioactivity of glycine reisolated from different tissues at a number of times after injection. After some variations during the first few days they found a long period of persistent differences in the specific radioactivities of reisolated glycine. They were led to conclude from this non-equilibration of glycine throughout the animal body that there was an absence of a single metabolic pool of the amino acid. A number of possible causes were listed to account for this lack of equilibration, including cell permeability barrier differences in the various tissues, active transport, free glycine levels in the tissues, the rates of protein synthesis and turnover, and the biosynthesis and metabolism of glycine itself. Restricting themselves to cat brain and liver, Berl et al. (1) used N^{15} -labelled substrates to follow glutamic acid metabolism to glutamine or aspartic acid and obtained data suggesting multicompartmentalization, with aspartic acid arising from a small, highly active reservoir of glutamate. Glutamine in the brain is also derived from a small reservoir of glutamate, but in the liver glutamine appears to come from a much larger pool of glutamic acid which mixes readily with tissue glutamate.

An investigation of the kinetic appearance of labelled amino acids in the intracellular amino acid pool and in cellular protein was undertaken under steady-state conditions in rat diaphragms and in isolated guinea pig and rabbit lymph-node cells (21). The ratios of the concentrations of labelled amino acids between the intra- and extracellular pools

approached equilibrium exponentially, while incorporation into protein was linear. A theoretical model was developed and the experimental data shown to fit the hypothesis that the intracellular amino acid pool is not an obligatory intermediate in protein synthesis. There must thus be more than one pool. One cannot distinguish yet between pools maintained separate by structural barriers from those effected by a chemical environment. Structural compartmentalization implies the restriction of the active pool of amino acids to particular sites (ribosomes, nuclei or mitochondria) known to be the locations of protein synthesis. A chemically separate pool might imply that incoming amino acids are immediately converted to S-RNA esters in preference to pre-existing intracellular amino acids, so that a free intracellular pool of amino acids engaged in protein synthesis may not exist. The origin of the distinction between the two sorts of amino acids would then lie in the nature of the preferential formation of S-RNA esters with the incoming molecules. Support for structural compartmentalization, with perhaps different metabolic characteristics in each compartment, comes from evidence on the intracellular localization of enzymes. Borst and Peeters (2), for instance, found in pig and ox heart that two separate glutamic-oxalacetic transaminases are present. One is found exclusively in the soluble cytoplasm while the other is confined to the mitochondria.

In micro-organisms an extensive study has been made of the amino acid pools by workers at the Carnegie Institution in Washington. In Candida utilis two pools were found (8). One of these, termed the "expandable pool", concentrates amino acids from the external medium. Its size is variable, being dependent on the concentration of the external amino acids, and amino acids in this pool exchange readily with those in the medium. This pool is sensitive to osmotic shock and loses much of its material if the cells are

removed from growth medium and placed in water. The second, or "internal", pool interconverts amino acids and selects them for protein synthesis. Metabolism of amino acids thus takes place solely in the internal pool. This reservoir maintains a constant size, and is affected neither by the concentration of amino acids in the medium, nor by osmotic shock. Amino acids are not exchanged with the medium or with the expandable pool, and those in this internal pool seem to be quite tightly bound. Similar results have been obtained in nucleic acid synthesis in C. utilis (7). In this case supplementation of the medium with nucleic acid bases leads to a concentrating pool which accumulates the bases internally to levels exceeding the external concentration. This pool then gives rise to a nucleotide pool which is always constant in size and which, in the absence of external bases, is derived from the sugar used as the carbon source. These two pools are analogous to the expandable and internal pools of amino acids.

An extension of amino acid pool studies to Escherichia coli (4) has shown that, at least for proline and valine, amino acids supplied outside the cell must pass through or equilibrate with the internal amino acid pool before incorporation into protein. It is possible, however, that internally synthesized amino acids may by-pass the internal pools, and this is supported by the failure of external lysine and aspartic acid, even at high concentrations, to substitute completely for the internally synthesized amino acid in protein synthesis (34). In the case of the incorporation of nucleic acid bases in RNA in E. coli it is known that there is a large by-pass flow, and that here too at least two pools of intermediates exist (23).

Compartmentalization, although incomplete, has also been found to exist for amines in neoplastic mast cells. These cells normally synthesize amines and also concentrate them from the medium; in both cases the amines are not metabolized but are eliminated from the cells. Green and Furano (14) observed with 5-hydroxytryptamine and histamine that cells which do contain endogenously produced amines also contain externally supplied amines in subcellular fractions which are normally devoid of endogenous amines. However, if the endogenous amines are completely absent, then external amines are found in those fractions normally containing endogenous amines. The amines from the two sources are therefore normally kept separated and accessibility of the endogenous amine pool for external amines depends on the concentration of endogenous amines already present. The authors have reviewed some examples of intracellular compartmentalization and the differing fates of substances originating inside or outside the cell (10). They point out that not only may the turnover rate of a compound depend on its site of origin, and the effect of drugs on metabolism differ if the metabolite originates inside or outside the cell, but the disposition of external substances may be affected by the intracellular level of the same substance derived from an endogenous source.

Gaizhoki (11) has shown that separate reservoirs of intracellular adenylic nucleotides coexist in the mitochondria and hyaloplasm of mouse hepatic cells and Ehrlich ascites carcinoma cells, and proposed that the diffusion of these compounds in and out of mitochondria as a result of changes in the permeability of the mitochondrial membrane may be a controlling factor in glycolysis. Mitochondria release adenylic nucleotides in isotonic solution, and this secretion stimulates glycolysis in the

hyaloplasm. In Ehrlich cells incubation of the mitochondria with hyaloplasm stimulates the swelling of the mitochondria and release of nucleotides. As the concentration of ATP in the hyaloplasm increases as a result of glycolytic activity, the mitochondria contract, cease releasing nucleotides, and glycolysis slows down to achieve a balanced state. Large molecules, such as pyridine nucleotides, are also unable to cross mitochondrial membranes. A good deal of the metabolic reduction of diphosphopyridine nucleotide is cytoplasmic (e.g. from glycolysis) yet reoxidation involves the electron transport system which is intramitochondrial and thus not directly accessible to reduced DPN. Schemes have been proposed of shuttle mechanisms between the intra- and extramitochondrial pools of DPN, utilizing either the dihydroxyacetone phosphate: α -glycerophosphate system or the acetoacetate: β -hydroxybutyrate system. These smaller molecules are able to cross the membrane barrier. Reduced DPN may reduce acetoacetate to β -hydroxybutyrate, simultaneously becoming reoxidized. β -Hydroxybutyrate then enters the mitochondria, there to be reoxidized to acetoacetate by intramitochondrial DPN. The latter is thereby reduced and is reoxidized via the cytochrome system to which it now has access (3). Conclusions consistent with this scheme were reached by Hoberman (16) as a result of his studies on the coupling of the oxidation of substrates to reductive biosynthesis. In rats, glycogen incorporated a highly significant quantity of deuterium from lactate-2- H^2 , less from malate-2:3- H^2 and none from lactate plus β -hydroxybutyrate-2:3- H^2 . Deuterium from these substrates was not incorporated into cholesterol or fatty acids. These results suggest a high degree of substrate specificity in the reductive synthesis of triose phosphate and therefore that intracellular pyridine nucleotide is kinetically

inhomogenous. α, α' -Deuterofumarate donates a significantly greater quantity of deuterium to glycogen than does malate-2:3- H^2 ; thus, endogenously formed malate is better used for glycogen synthesis than is externally supplied malate. In the respiratory chain itself in liver mitochondria there seems to be no compartmentalization (20) and, at least as far as the succinic and choline oxidase systems are concerned, these sequences are interlinked at and above the oxidation level of cytochrome C_1 . It appears that two or more dehydrogenases may be linked to a given cytochrome chain and there is intercommunication (i.e. electron transport) among all the cytochrome chains in a given mitochondrion.

Carbon metabolism through glycolysis, the pentose phosphate cycle and the tricarboxylic acid cycle has received the attention of several investigators. Among the most elegant of these studies are those reported by Shaw and Stadie (38,39) for rat diaphragm muscle. They studied the ability of the muscle to incorporate radiocarbon from external substrates into glycogen and into a number of glycolytic intermediates. The influence of insulin on the system was investigated and the use of this hormone enabled conclusions to be drawn concerning the localization of some of the metabolic activities. In a phosphate buffer it was shown that medium glucose may be metabolized by way of glucose-6-phosphate and glucose-1-phosphate to glycogen. Fructose-1:6-diphosphate is not formed from the glucose-6-phosphate. Insulin stimulates the synthesis of glycogen from glucose and increases the turnover of the two glucose phosphates. Moreover, these sugar phosphates do not leave the interior of the diaphragm and, if added to the medium, neither glucose-6-phosphate nor glucose-1-

phosphate contribute carbon to glycogen. Glucose phosphates present in the medium do not mix with the chemically identical glucose phosphates on the biosynthetic route to glycogen.

By a second, glycolytic, pathway glucose in the medium is converted via glucose-6-phosphate, fructose-1:6-diphosphate, etc., to lactic acid. This pool of glucose-6-phosphate was not the same as the one on the route to glycogen. When glucose-6-phosphate and fructose-1:6-diphosphate (but not glucose-1-phosphate) are added to the medium they enter the glycolytic sequence and interchange with the chemically identical phosphates formed from glucose in the medium. It was shown that this conversion of glucose to lactate via pools of sugar phosphates which could exchange with sugar phosphates in the medium was not due to the leaching of enzymes from the cell. Insulin did not influence the formation of lactic acid from glucose. These data led to the conclusion that two sites of glucose metabolism exist: an intracellular site leading to glycogen (which is known to be formed inside the cell), and an extracellular locus of glycolytic activity. Since insulin action is believed to be related to the transport of glucose into the cell, an explanation was provided for the stimulation of (intracellular) glycogen formation from glucose by insulin, which had no effect on the (extracellular) metabolism of glucose to lactic acid.

When the suspending medium was changed from a phosphate buffer to a bicarbonate buffer it was found that insulin not only affected glycogen synthesis but also had some stimulating effect on the formation of lactate from glucose. In bicarbonate buffer it was possible to detect labelled fructose-1:6-diphosphate from glucose-C¹⁴ inside as well as outside the cell, though there was still no exchange of the phosphate esters across the cell membrane. Finally, it was found that the intracellular phospho-

fructokinase was inactive in the absence of bicarbonate. There are thus two glycolysis pathways coexisting in rat diaphragm muscle. One of these lies inside the cell as evidenced firstly, by the lack of equilibration between phosphates formed metabolically and those added to the medium, and secondly, by the fact that glycogen is certainly laid down within the cell. The other pathway lies outside the cell, perhaps on the surface; this is demonstrated by the mixing between medium phosphates and those formed metabolically from glucose.

Another example of compartmentalization in carbohydrate metabolism was found in the fungus Zygorhynchus moelleri. Kinetic studies made of the incorporation of radiocarbon from labelled bicarbonate in the presence of unlabelled glucose showed that the first appearance of label in a phosphate ester was into 6-phosphogluconate, about four seconds after supplying the bicarbonate (29). There was a rapid formation of tricarboxylic acid cycle intermediates, and eventually radiocarbon appeared in 3-phosphoglyceric acid (indicating a reversal of glycolysis) and in hexose monophosphates, but none appeared in the sugar diphosphates even after thirty minutes of incubation. Since the diphosphates did not become labelled before the monophosphates, the latter probably arose by reduction of phosphogluconate. From studies of glucose metabolism, however, it was known that glucose rapidly gave rise to hexose diphosphates via the monophosphates (26). The fact that hexose monophosphate formed by reduction of phosphogluconate did not rapidly form hexose diphosphate suggests that this pool of hexose monophosphate is different from the one formed from glucose.

Further attempts to study compartmentalization in carbohydrate metabolism were made in ascites tumour cells of mice (27). Here it was reasoned that if compartmentalization does not exist, then irrespective

of the origin of a substance entering a sequence of metabolic reactions, the proportion of the quantities of the several products of the sequence will be the same, provided that the physiological and biochemical state of the cell does not vary during the experiment. In other words, the products of metabolism of a substrate will be in the same proportion whether the substrate is supplied in the medium or generated endogenously within the cell. As the cell must be kept chemically constant, the substrate must be simultaneously added to the medium and be produced inside the cell. However, the fate of the substrate presented to the cell in each of the two ways may be studied separately by arranging that in one incubation vessel the substrate originating in one way, but not in the other, is labelled isotopically. In another vessel, chemically identical, the labelling pattern is reversed. If there is compartmentalization, then the possibility arises that the substrates originating from different sources may enter different compartments, and give rise to different distributions of product compounds. Again, such differences might be found by differential labelling experiments.

This technique has been applied to glycolysis in the ascites cells. Five substrates were supplied simultaneously, but in each separate vessel only one was labelled: glucose, glucose-6-phosphate, fructose, 3-phosphoglyceric acid, and lactic acid. Thus it was possible to compare the behaviour, for example, of phosphoglyceric acid produced metabolically from glucose via glucose-6-phosphate and fructose-6-phosphate, from glucose-6-phosphate via fructose-6-phosphate, from free fructose via fructose-6-phosphate, and from phosphoglyceric acid added to the medium. Preliminary results have suggested that as many as four compartments, each containing all or part of glycolysis, may be present together in the ascites cell.

Of the five substrates added to the medium, only glucose and lactic acid seem to share the same metabolic compartment -- i.e. lactate made from glucose mixes with lactate in the medium. Fructose, glucose-6-phosphate and phosphoglycerate all appear to be metabolized, at least in the early stages, separately from one another and from glucose and lactate.

Compartmentalization of the acids of the tricarboxylic acid cycle in plant tissues has recently been investigated by MacLennan *et al.* (24). Having demonstrated that acetate was metabolized via the cycle, they studied the incorporation of radiocarbon from acetate- 1-C^{14} into a number of carboxylic and amino acids, and into respired carbon dioxide. Even after the specific radioactivity of the carbon dioxide had become constant, indicating that the pools of intermediates between acetate and carbon dioxide had become equilibrated with acetate carbon, the specific activities of the Krebs cycle acids after extraction from the plants showed great differences, demonstrating the existence of unlabelled pools of these acids in addition to those containing radioactivity. Extraction of the acids from the plants, of course, destroys any compartmental separation. It was calculated that large amounts of the Krebs cycle acids did not turn over when the acetate was supplied. This work provides one of the few cases in which it was possible to make some degree of correlation between pool separation and structural features. In maize roots increasing age brings with it increasing intracellular vacuolation. At the same time there is a marked increase in the relative amounts of the acids found in inactive reservoirs outside the rapidly turning-over pools, with the implication that the inactive reservoirs may be located within the vacuoles.

Pette and his coworkers (30,31) have studied the specific proportions of a number of enzymes in subcellular fractions which may be pre-

sumed to correspond to different compartments. They found, for example, that in mitochondria from a number of different tissues and from different species of animals, more or less constant amounts of malic dehydrogenase, glutamic dehydrogenase, TPN-linked isocitric dehydrogenase, glutamic-oxalacetic transaminase, succinic dehydrogenase, glycerol-1-phosphate oxidase and pyruvic oxidase were present compared with the quantity of cytochrome C (31). Similar constant proportionalities, again from many tissues, were found in the extramitochondrial fraction for the enzymes triose phosphate isomerase, 3-phosphoglycerinaldehyde dehydrogenase, 3-phosphoglyceric kinase, phosphoglycerate mutase and enolase (30). They found, however, that though the ratios of activities of functionally closely related enzymes may remain roughly constant in different cellular compartments, the concentrations of the related enzymes on a weight basis may vary widely in the different compartments (32). Thus the total enzymic capabilities of different compartments may exhibit great variations. This points up the possibility, made use of by Moses, Chang and Lonberg-Holm (27), that different proportions of metabolic products may be expected in each compartment, although qualitatively the same chemical sequences of reactions occur at each locus.

The fact that different forms of some enzymes have been isolated from certain tissues (17) does not necessarily mean that they are located in separate compartments. Stadtman (41) has discussed the presence in Escherichia coli of three different versions (isozymes) of aspartokinase, all of which catalyze the reaction between aspartic acid and adenosine triphosphate. Aspartate is known in E. coli to be the parent substance of three other protein amino acids: lysine, methionine and threonine. The biosynthetic pathways of all three are common from aspartic acid

through aspartyl phosphate to aspartic semialdehyde. From that point on the pathway of lysine biosynthesis branches away. Methionine and threonine biosyntheses continue along a common path to homoserine and then they diverge. Control of the biosyntheses of these three amino acids is achieved by both feedback inhibition (retro-inhibition) and repression exerted by the three end-product amino acids on various enzymes along the routes leading to each, and including aspartokinase, an enzyme common to all three. It can be shown physically and enzymologically that three different versions of aspartokinase are present. In addition to showing some physical differences, these enzymes exhibit a varying response to feedback inhibition and repression by lysine, methionine and threonine. Thus, one enzyme is inhibited and its synthesis repressed by lysine, though the other two isozymes are not affected. The second enzyme is specifically inhibited and repressed by threonine, while the third enzyme is unaffected by all three amino acids. Genetic evidence has been interpreted to indicate that the three aspartokinases cannot be performing similar roles in parallel, compartmentalized pathways, but must feed into a common pool of aspartyl phosphate. In an auxotrophic mutant of *E. coli*, requiring threonine, methionine, and diaminopimelic acid (a lysine precursor), the requirement for all three amino acids was shown to be the consequence of a single mutated locus resulting in the inability of the mutant to synthesize aspartic semicaldehyde dehydrogenase (13). This has been held to preclude the existence of multiple enzymes catalyzing the conversion of aspartyl phosphate to aspartic semialdehyde, implying, in turn, that one pool only of each of these substances exists.

If, in conclusion, we consider data from different areas of biochemistry, we can see that the evidence supporting the concept of intracellular

compartmentalization is accumulating from many sources. Until now most investigations of the phenomenon have exploited some peculiar characteristic of the biological system to lead to conclusions relating to compartmental organization. Individual species have not been studied systematically and no attempts have so far been made to follow and interrelate the consequences of compartmentalization in consecutive series of biochemical reactions. It might be possible eventually to build up a picture of metabolic activity which describes not just the sum of all the possible chemical interconversions, but relates the reactions undergone by a given compound to its biological origin. Even in those systems which have been shown to behave in a compartmentalized manner, almost nothing is known about the physical sites of the various pools. Much obviously remains to be done in this direction; at the present time we can be fairly certain that intramitochondrial events, for example, are to some degree separated from those occurring outside these organelles, but we do not know whether subcompartments might not also exist inside the mitochondria themselves, perhaps as surface localizations of groups of enzymes on the walls of the cristae. It is interesting that Pontecorvo (33) has predicted on theoretical grounds that the nucleus will be found not to exhibit compartmentalization, but whether or not individual nuclei, mitochondria, or ribosomes do themselves show this effect we need not doubt that compartmentalization is a widespread property of biological systems and is in all likelihood intimately connected with the basic events of cellular organization, regulation, and differentiation.

REFERENCES

1. Berl, S., G. Takagaki, D. D. Clarke, and H. Waelch: *J. Biol. Chem.* 237, 2562-2569 (1962).
2. Borst, P., and E. M. Peeters: *Biochim. et Biophys. Acta* 54, 188-189 (1961).
3. Boxer, G. E., and T. M. Devlin: *Science* 134, 1495-1501 (1961).
4. Britten, R. J., and F. T. McClure: *Bact. Revs.* 26, 292-335 (1962).
5. Campbell, J. J. R., L. A. Hogg, and G. A. Strasdine: *J. Bact.* 83, 1155-1160 (1962).
6. Conway, E. J., and M. Downey: *Intern. Congr. Biochem., Abstrs. of Communs., 1st. Congr. Cambridge, Engl.* 305 (1949).
7. Cowie, D. B., and E. T. Bolton: *Biochim. et Biophys. Acta*, 25, 292-298 (1957).
8. Cowie, D. B., and F. T. McClure: *Biochim. et Biophys. Acta* 31, 236-245 (1959).
9. Dormer, K. J., and H. E. Street: *Ann. Bot. Lond.* N.S. 13, 199-217 (1949).
10. Furano, A. V., and J. P. Green: *Nature, Lond.* 199, 380-381 (1963).
11. Gaizhoki, V. A.: *Biokhimiya* 26, 926-933 (1961).
12. Garfinkel, D., and B. Hess: *J. Biol. Chem.* 239, 971-983 (1964).
13. Gilvarg, C.: *J. Biol. Chem.* 237, 482-484 (1962).
14. Green, J. P., and A. V. Furano: *Biochem. Pharmacol.* 11, 1049-1053 (1962).
15. Heber, U., and J. Willenbrink: *Biochim. et Biophys. Acta* 82, 313-324 (1964).

16. Hoberman, H. D.: *J. Biol. Chem.* 232, 9-16 (1958).
17. Kaplan, N. O.: *Bact. Revs.* 27, 155-169 (1963).
18. Karassevitch, Y., and H. de Robichon-Szulmajster: *Biochim. et Biophys. Acta*, 73, 414-426 (1963).
19. Kearney, P. C., and N. E. Tolbert: *Arch. Biochem. Biophys.* 98, 164-171 (1962).
20. Kimura, T., and T. P. Singer: *Nature, Lond.* 184, 791-793 (1959).
21. Kipnis, D. M., E. Reiss, and E. Helmreich: *Biochim. et Biophys. Acta* 51, 519-524 (1961).
22. Lonberg-Holm, K. K.: University of California Lawrence Radiation Laboratory Report UERL-10357 (1962).
23. McCarthy, B. J., and R. J. Britten: *Biophys. J.* 2, 35-47 (1962).
24. MacLennan, D. H., H. Beevers, and J. L. Harley: *Biochem. J.* 89, 316-327 (1963).
25. Monod, J., J.-P. Changeux, and F. Jacob: *J. mol. Biol.* 6, 306-329 (1963).
26. Moses, V.: *J. gen. Microbiol.* 20, 184-196 (1959).
27. Moses, V., Julia J. Chang, and K. K. Lonberg-Holm: *Intern. Congr. Biochem., Abstrs. of Communs, 6th Congr., New York, U.S.A. IX-59* (1964).
28. Moses, V., O. Holm-Hansen, J. A. Bassham, and M. Calvin: *J. mol. Biol.* 1, 21-29 (1959).
29. Moses, V., O. Holm-Hansen, and M. Calvin: *J. Bact.* 77, 70-78 (1959).
30. Pette, D., W. Luh, and T. Bücher: *Biochem. Biophys. Res. Communs.* 7, 419-424 (1962).
31. Pette, D., M. Klingenberg, and T. Bücher: *Biochem. Biophys. Res. Communs.* 7, 425-429 (1962).
32. Pette, D., and W. Luh: *Biochem. Biophys. Res. Communs.* 8, 283-287 (1962).
33. Pontecorvo, G.: *Proc. Roy. Soc.* 158B, 1-23 (1963).

34. Roberts, R. B., P. H. Abelson, D. B. Cowie, E. T. Bolton, and R. J. Britten: Studies of Biosynthesis in Escherichia coli, Washington, D.C.: Carnegie Institution of Washington Publication 607, 1955.
35. Robichon-Szulmajster, H. de, and D. Corrivaux: Biochim. et Biophys. Acta 73, 248-256 (1963).
36. Rothstein, A., D. H. Jennings, C. Demis, and M. Bruce: Biochem. J. 71, 99-106 (1959).
37. Schapira, G., J.-C. Dreyfus, J. Kruh, D. Labie, and P. Padiou: Proc. 2nd U.N. Intern. Conf. on Peaceful Uses of Atomic Energy 25, 21-24 (1958).
38. Shaw, W. N., and W. C. Stadie: J. biol. Chem. 227, 155-134 (1957).
39. Shaw, W. N., and W. C. Stadie: J. biol. Chem. 234, 2491-2496 (1959).
40. Smith, D.C., J. A. Bassham, and M. Kirk: Biochim. et Biophys. Acta 48, 299-313 (1961).
41. Stadtman, E. R.: Bact. Revs. 27, 170-181 (1963).
42. Stier, T.J.B., and J. N. Stannard: J. gen. Physiol. 19, 479-494 (1936).
43. Sutton, D. D., and J. O. Lampen: Biochim. et Biophys. Acta 56, 303-312 (1962).
44. Wilkes, G. B., and E. T. Palmer: J. gen. Physiol. 16, 233-242 (1932).

This report was prepared as an account of Government sponsored work. Neither the United States, nor the Commission, nor any person acting on behalf of the Commission:

- A. Makes any warranty or representation, expressed or implied, with respect to the accuracy, completeness, or usefulness of the information contained in this report, or that the use of any information, apparatus, method, or process disclosed in this report may not infringe privately owned rights; or
- B. Assumes any liabilities with respect to the use of, or for damages resulting from the use of any information, apparatus, method, or process disclosed in this report.

As used in the above, "person acting on behalf of the Commission" includes any employee or contractor of the Commission, or employee of such contractor, to the extent that such employee or contractor of the Commission, or employee of such contractor prepares, disseminates, or provides access to, any information pursuant to his employment or contract with the Commission, or his employment with such contractor.

