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The Interconversion of Amino Acids after their Incorporation into Haemoglobin and Myosin

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Kruh, Dreyfus, Schapira & Padiou (1957) have shown that haemoglobin is not uniformly labelled when either radioactive glycine or phenylalanine is injected (Kruh, Dreyfus & Schapira, 1960), but that the labelling becomes uniform when the red blood cells become older. These results have suggested several hypotheses; one of them was that haemoglobin does not remain inert and undergoes intramolecular changes during the life of the red cells.

It has been observed that myosin is sufficiently stable to reflect the life span of the myofibrils (Dreyfus, Kruh & Schapira, 1960). Comparison of the specific activities of glycine and serine, after the injection of radioactive glycine, and of the specific activities of phenylalanine and tyrosine, after the injection of radioactive phenylalanine, has suggested that a conversion of glycine into serine and of phenylalanine into tyrosine could have occurred after the incorporation of the precursor amino acids, during the life of the red blood cells and of the myofibrils (a preliminary report of these observations has been published; Schapira, Dreyfus & Kruh, 1958). Similar conversion has been shown to occur in collagen (Stetten, 1949; Piez & Likins, 1957; Van Slyke & Sinex, 1958; Sinex, Van Slyke & Christman, 1959; Robertson, Hiwett & Herman, 1959; Green & Lowther, 1959;

Chvapil & Cmucha, 1960). Our study is favourably influenced by the fact that haemoglobin and myosin are not renewed or very slowly renewed, which makes it possible to exclude rapid exchange between bound and free amino acids.

Preliminary experiments have shown that when the animals were fed a normal diet the ratios of the specific activities of glycine to serine in myosin rapidly became unity after the injection of radioactive glycine. Besides, Neuberger (1951), using rats and rabbits on a normal diet, found a ratio between specific activities of protoporphyrin and globin glycine constant with time. It is likely that the equilibration had already occurred after 2 days. On the other hand, these ratios approached unity much more gradually when the rats were fed a low-protein diet; the interconversion of amino acids after their incorporation in protein is thus more readily followed.

METHODS

Animals. The experiments were carried out with white rats of Wistar strain, weighing between 200 and 300 g. They were given a diet poor in protein, i.e. containing 8% of a mixture of animal and vegetable protein. The rats showed neither loss of weight nor anaemia. An estimation of the haemoglobin was made in each experiment. Twenty-four rats were injected intraperitoneally with [2-¹⁴C]glycine and [3-¹⁴C]phenylalanine at a dosage of 4 μ C

of each for 100 g. of body weight. The rats were killed by bleeding, between the 2nd and the 62nd day after the injection. Two rats were used in each experiment.

Preparation of material and expression of results

Blood proteins. Blood was collected in syringes containing heparin. After centrifuging, the plasma proteins were precipitated by addition of one-half volume of 20% (w/v) trichloroacetic acid. The precipitate was washed twice with 7% (w/v) trichloroacetic acid, twice with acetone, and once with ether. The red blood cells were washed three times with an iso-osmotic solution of NaCl. They were haemolysed by the addition of 2 vol. of water, and brought to pH 9.0 with 2N-NH₃ solution to facilitate the extraction of haemoglobin. The haemolysate was treated with 0.5 vol. of cold toluene, kept in the cold overnight and then filtered. In one series of experiments haemoglobin was crystallized by the technique of Drabkin (1949); the results were the same as with the former method. Haem was split from globin by adding 10 vol. of acetone containing 1% of HCl (Anson & Mirsky, 1930); the resulting haemin was precipitated by the addition of water and evaporation at 50°. The precipitate was dissolved in pyridine, filtered into copper planchets and evaporated under an infrared lamp. The pyridine haemochromogen was weighed, and correction for thickness was made. The specific activities are expressed in counts/min./μmole of the pyridine haemochromogen, divided by 8, since eight molecules of glycine are used for the biosynthesis of one residue of haem. The radioactivities were measured in the gas-flow counter.

The globin precipitate was thoroughly washed with acetone.

Myosin. About 60 g. of muscle was obtained and homogenized in a meat grinder. Myosin was prepared according to Szent-Gyorgyi (1951). Its purity was established by moving-boundary electrophoresis, which always showed one symmetrical peak. The absence of actomyosin was shown by the absence of decrease of the viscosity after the addition of ATP. Myosin was precipitated by trichloroacetic acid and treated as described by Dreyfus *et al.* (1960).

Liver proteins. A portion of the liver was homogenized in a Potter-Elvehjem homogenizer with 5 vol. of 10% (w/v) trichloroacetic acid. The proteins were precipitated by addition of 20% (w/v) trichloroacetic acid. The precipitate was treated twice with 5% (w/v) trichloroacetic acid, twice with acetone and once with ether.

Hydrolysis of proteins, separation of the amino acids and measurement of the specific activities

The proteins were dried to constant weight at 60°, and ground in an agate mortar. They were uniformly spread on copper planchets of 2.85 cm.² surface. The deposit was assumed to be of infinite thickness. Their radioactivities were measured in a Geiger-Müller counter, and are expressed as counts/min./mg.

The hydrolyses were carried out in 1000 vol. of 6N-HCl in an air bath at 112° for 48 hr.; HCl was evaporated *in vacuo* and the hydrolysates were freed from HCl by addition of water and evaporation to dryness six times. The amino acids were fractionated as follows.

Glycine and serine were isolated as DNP derivatives on a Celite column according to the modified method of Perrone (1951) (Kruh *et al.* 1957). The purity of the DNP

amino acids was checked by paper chromatography according to Levy (1954) and by melting point.

The DNP derivatives were estimated by spectrophotometry at 360 mμ after dilution of a sample in 2% NaHCO₃ solution. Another portion was evaporated, dissolved in methanol and evaporated on copper planchets of 2.85 cm.² surface, on which a lens paper was fixed with silicone for measuring the radioactivity. Phenylalanine and tyrosine were adsorbed on a charcoal column, eluted and fractionated on a Dowex 50 ion-exchange column, according to the technique of Watchel & Cassidy (1943) modified by Velick (1956) and by Kruh *et al.* (1960). The purity of the amino acids was checked by paper chromatography. The estimations were carried out after dilution of a sample in N-NH₃ solution by spectrophotometry at 260 mμ for phenylalanine and at 280 mμ for tyrosine. Another sample was evaporated on copper planchets.

The radioactivities were measured in a gas-flow counter with a statistical error of ±2%; corrections for thickness were made, and the results are expressed as counts/min./μmole.

Estimation of C-3 of serine. In order to elucidate the mechanism of the conversion of glycine into serine, we have isolated C-3 of the serine and measured its specific activity. We have used a modification of the technique of Rees (1946). (This technique was based on the ability of HIO₄ to oxidize the C-3 to formaldehyde, which was precipitated by addition of dimedone.) Protein hydrolysate (200 mg.) was dissolved in 5 ml. of water and the solution poured on an ion-exchange column consisting of Permutite 50 (H⁺ form; 100–200 mesh).

The carbohydrates that react with HIO₄ were eluted with water and amino acids were eluted with 2N-NH₃ solution. Ammonia was evaporated *in vacuo* and the hydrolysate was dissolved in 1 ml. of water and treated with periodate according to Rees (1946). After eliminating the acetaldehyde arising from threonine by aeration, the formaldehyde was steam-distilled in a Parnas apparatus (Parnas & Wagner, 1921) into the dimedone solution. After centrifuging, the precipitate was dissolved in 2N-NH₃ solution and reprecipitated with acetic acid; this procedure was repeated twice. Finally it was dissolved in 2N-NH₃ solution, evaporated on a copper planchet and weighed, and its radioactivity measured, results being expressed as counts/min./μg. atom of C.

The following control experiments were carried out. Addition of threonine to radioactive serine did not decrease the specific activity of the formaldehyde, showing that threonine was completely eliminated in the first aeration. The radioactive serine was divided into two portions: one was diluted with non-radioactive serine and the other was diluted with a protein hydrolysate then isolated as DNP-serine and counted; the ratio of the specific activities of C-3 of serine/DNP-serine was the same in both cases (Table 1); only serine was involved in the reaction. When the reaction was carried out with carboxyl-labelled serine the formaldehyde was not labelled.

Estimation of tyrosine. This was performed according to Fraenkel-Conrat & Singer (1956) with some modification.

Samples (5 mg.) of globin or myosin were hydrolysed in evacuated sealed tubes with 1 ml. of constant-boiling twice-redistilled HCl for 48 hr. at 108°. After evaporation of the HCl and five subsequent additions of water, the residues were dissolved in 0.5 ml. of water.

Samples (50 and 100 μ l.) were placed on Whatman no. 1 paper sheets, interspaced with smaller portions and standard amino acid solution at comparable concentrations. Amounts of 30 μ g. were sufficient for analysis. The chromatograms were developed with butanol-acetic acid-water (250:60:250, by vol.; upper phase) and were dried and re-run twice. The length of the run was 30 cm.

The detection and elution were performed as by Fraenkel-Conrat & Singer (1956).

Tyrosine was estimated according to Udenfriend & Cooper (1952) as modified by Maisin & Lindenberg (1957).

RESULTS

Haemoglobin. The curve of the specific radioactivity of haemin and of globin plotted against time reaches a plateau followed by a decrease after the 40th day, which permits calculation of a life span of 45 days for the red cells (Fig. 1).

The specific activity of the glycine in globin reaches a maximum within 5 days, then decreases slowly, until the 30th day and more rapidly thereafter. That of serine, by contrast, increases until the 30th day, when it reaches the same value as that of glycine (Fig. 2).

Table 1. *Control experiment to check the isolation of C-3 of serine*

Comparison of the specific activities of the isolated C-3 and the total serine (prepared DNP derivative) after dilution with pure serine and with a protein hydrolysate.

	C-3 of serine	DNP- serine	Ratio
Dilution with serine	38 ± 2	30	1.27 ± 0.07
Dilution with a protein hydrolysate	54 ± 3	39	1.35 ± 0.08

Fig. 1. Variation with time of the specific activities of globin (O) and of haemin (Δ). The globin was labelled by injection of [14 C]glycine and [14 C]phenylalanine.

The specific activity of the phenylalanine in globin increases till the 20th day, remains as a plateau and begins to decrease only after the 45th day. That of tyrosine increases till the 30th day, when it reaches that of phenylalanine (Fig. 3).

The slight scatter, due to the use of different animals, disappears if we consider the values of the ratios of specific activities of serine/glycine and tyrosine/phenylalanine in the same animal. In this case, as shown in Fig. 4, there is a regularly ascending curve, culminating on the 30th day as a plateau in both cases.

If we consider not the specific activities of the amino acids but their total activities (by multiplying the specific activity of each amino acid by its relative amount in the protein) we observe that

Fig. 2. Variation with time of the specific activities of glycine (●) and of serine (□) incorporated into haemoglobin.

Fig. 3. Variation with time of the specific activities of phenylalanine (▲) and of tyrosine (■) incorporated into haemoglobin.

the radioactivity of the globin powder has a time curve parallel to that of the radioactivities of the four amino acids. Their ratio is plateau-shaped (Fig. 5) and the sum of the amino acids accounts for 90–100 % of the radioactivity of the protein.

Further, the curves of the radioactivities of glycine plus serine and phenylalanine plus tyrosine are both plateau-shaped, although they cannot be completely superposed, since glycine is incorporated faster than phenylalanine (Fig. 6).

Myosin. The curve of the radioactivity of myosin plotted against time shows a plateau

between the 2nd and the 25th days: it then decreases rapidly until the end of the experiment (62 days). This time course is quite analogous to that of globin and makes it possible to demonstrate a metabolic inertness of myosin and a 'life span' of the myofibril comparable with that of the red cell. It confirms our previous observations on the life span of the myofibril (approximately 30 days) (Fig. 7) (Dreyfus *et al.* 1960).

The specific activity of the myosin glycine reaches its maximum in the first sample and later decreases regularly. That of serine increases until the 15th day and then decreases (Fig. 7). The ratio of the specific activities of serine/glycine increases progressively but reaches unity only after 40 days (Fig. 9).

The specific activity of the phenylalanine of myosin has the same characteristics as that of globin. The plateau is slightly descending until the 25th day, when it decreases. The shape of the curve of phenylalanine specific activity gives us the best evidence for the reality of a 'life span' of myosin (Fig. 8). Tyrosine has a very low specific activity on the third day, but it increases rapidly and reaches 90 % of that of phenylalanine within 11 days, then remains unchanged until the 25th day and later decreases like that of phenylalanine without any delay between them. The ratio of specific activities of tyrosine/phenylalanine increases much more rapidly than that of the specific activities of serine/glycine (Fig. 9) and that in the globin (Fig. 4).

The ratio of the sum of the activities of the four amino acids to the radioactivity of myosin is plateau-shaped between the 3rd and the 40th day. Then it decreases, probably because of progressive

Fig. 4. Variation with time of the ratios of specific activities of serine/glycine (□) and tyrosine/phenylalanine (●) from globin.

Fig. 5. Variation with time of the ratio (total activities of glycine + serine + phenylalanine + tyrosine)/radioactivity of globin. The radioactivity of globin was expressed in counts/min. The total activity of each amino acid was obtained by multiplying its specific activity by its relative amount in globin.

Fig. 6. Variation with time of the total activities of glycine + serine (Δ) and of phenylalanine + tyrosine (●) per milligram of globin. The total activity of each amino acid was obtained by multiplying its specific activity by its relative amount in globin.

Fig. 7. Variation with time of the radioactivity of myosin (○) and of the specific activities of glycine (●) and serine (□) incorporated into myosin.

Fig. 8. Variation with time of the specific activities of phenylalanine (●) and tyrosine (Δ) incorporated into myosin.

Fig. 9. Variation with time of the ratios of specific activities of serine/glycine (□) and tyrosine/phenylalanine (●) from myosin.

Fig. 10. Variation with time of the ratio (total activities of glycine + serine + phenylalanine + tyrosine)/radioactivity of myosine. The radioactivity of myosin was expressed in counts/min., the total activity of each amino acid was obtained by multiplying its specific activity by its relative amount in myosin.

transformations into other amino acids (Fig. 10). The sum of the radioactivities of glycine plus serine gives a plateau between the 3rd and 20th days. The sum of the radioactivities of phenylalanine plus tyrosine gives a plateau between the 10th and 20th days (Fig. 11). The sum of the activities of the amino acids accounts for more than the activity of the myosin (110–120 %).

Fig. 11. Variation with time of the total activities of glycine + serine (Δ) and of phenylalanine + tyrosine (●)/mg. of myosin with time. The total activity of each amino acid was obtained by multiplying its specific activity by its relative amount in myosin.

This discrepancy may be due to experimental error, which is the result of five independent determinations. It may also be due to the fact that we have assumed for the rat the same amino acid concentration as for the rabbit, which may be slightly in error.

In a few experiments, the specific activity of aspartic and glutamic acid and of threonine isolated from globin and myosin hydrolysates by a DNP derivative has been measured: threonine was inactive, aspartic and glutamic acid had a specific activity about 10 % or less of that of glycine; these specific activities show no tendency to increase with the duration of the experiment.

Liver. The specific activity of the amino acids has been determined in the plasma proteins and in the whole proteins of liver at the earliest time (3 days). In a previous series of experiments, the specific activity was measured after 1 day for glycine and serine. In all cases the ratio of the specific activities of serine/glycine was 1 or more, and 1 for the ratio of the specific activities of tyrosine/phenylalanine. Thus at all times during the experiments, the proteins made from the hepatic pool of amino acids had a ratio of the specific activity of serine to glycine and of tyrosine to phenylalanine approaching unity.

DISCUSSION

Haemoglobin

After injection of glycine, the specific activities of haem and globin remain constant for 33 days, but the constancy of the radioactivity of the globin is the result of the reciprocal opposite variation of the specific activity of glycine and serine. When the specific activity of globin glycine decreases, specific activity of globin serine increases; accordingly the ratio of specific activities of haem/(globin glycine)

constantly increases, whereas the corresponding ratio haem/globin is constant. The curve of the total activity of globin glycine and serine (specific activity $\times$ percentage of glycine + specific activity $\times$ percentage of serine) is plateau-shaped; so is the curve of haem. The same pool of labelled glycine is used as precursor for the biosynthesis of haem and of globin, which both present a radioactivity constant with time.

Equations derived to compute the rate of turnover of the amino acids in globin would give results too imprecise, owing to the small difference in the specific activity of the amino acids at the beginning and the end of the experiment.

Therefore if globin is in a dynamic equilibrium, by contrast the haem moiety is, apparently, metabolically stable. However, the haem might be temporarily liberated during the renewal of the globin moiety, either in a free state, or combined to a globin fragment.

Myosin

The results obtained with myosin are essentially the same as those obtained with globin. The radioactivity of the myosin remains constant for 25 days, then it decreases. This plateau of the radioactivity has been attributed to a life span of the myofibril by Dreyfus, Kruh & Schapira (1960). The curve of the total activity of myosin glycine and serine remains constant during the first 20 days, it decreases slightly the 31st day, and afterwards it decreases rapidly. On the other hand, the specific activity of myosin glycine decreases and that of myosin serine increases; the interpretation is the same as for globin. With phenylalanine, the increase of the specific activity of tyrosine occurs during a much longer time than the increase of the specific activity of phenylalanine, but the decrease occurs on the 30th day for both amino acids (Fig. 8).

Conversion of glycine into serine and phenylalanine into tyrosine

The apparent equilibration of the specific activity of glycine and serine may be due to several mechanisms. It involves in any case the fixation on the glycine molecule of a C₁ fragment, but the latter can come from two sources: C-3 of serine (which may arise from the medium), or in part from C-2 of glycine. If this were so the radioactive serine would be a mixture of [3-¹⁴C]- and [2-¹⁴C]-serine. We have determined the specific activity of the C-3 of serine. In all cases this specific activity proved to be low. C-3 comes therefore essentially from a source other than radioactive glycine and the ratio of the specific activities of serine/glycine actually describes an equal specific activity of the C-2 of glycine and serine.

This transformation is reversible: it may occur in

several steps. The conversion in a free state is very rapid in liver (Winnick, Moring-Claesson & Greenberg, 1948); it is much slower in reticulocytes: after an incubation for 4 hr. only 14 % of labelled glycine is converted into serine (Udenfriend & Cooper, 1952).

The acquisition of radioactivity by the globin serine appears to be definitely biphasic. The early-labelled serine receives contributions from extra-globular (perhaps hepatic) serine, but after about a week when the cells have left the marrow this process is unimportant and the slower process of intraglobular equilibration thus becomes apparent.

The conversion of free phenylalanine into tyrosine has been shown to be rapid in the liver (Winnick *et al.* 1948); it does not occur, or is very slow, in reticulocytes during incubation for 4 hr. (Kruh *et al.* 1960), but this does not exclude a very slow rate of conversion during the span of life of red cells, which might be impossible to detect in ordinary enzymic assay.

Exchanges between phenylalanine and tyrosine may be excluded: when we injected radioactive tyrosine into rats, phenylalanine did not become radioactive even 1 month later, which excluded this mechanism.

Hypothesis about the molecular mechanisms of conversion of amino acid in bound state

Time of the conversion. The conversion of glycine into serine is known to occur in the free state, in reticulocytes for example (Borsook, Deasy, Haagen-Smit, Keighley & Lowy, 1952); we have to establish that such a conversion before the incorporation cannot explain the results. If only such a conversion were involved we would have to suppose that the red cells synthesized after the injection of [¹⁴C]glycine would be rich in radioactive glycine; the red cells synthesized later would be rather rich in ¹⁴C from serine. In such a case, the life span of the two types of red cells being the same, the decrease of the specific activity of serine should occur later than the decrease of the specific activity of glycine. This is not the case; the decrease of the specific activity of the two incorporated amino acids occurs at the same time.

Our results exclude the hypothesis of a conversion of glycine into serine before the incorporation of the former amino acid.

The same phenomenon has been observed with phenylalanine and tyrosine: nevertheless, the evolution of the specific activity of these amino acids is not the same as with glycine and serine. Even after the seventh day after the injection of [¹⁴C]phenylalanine there is a slight increase of the specific activity of the incorporated phenylalanine, and a rapid increase of the specific activity of the incorporated tyrosine until the 20th day. But

thereafter the specific activity of the two amino acids remains the same until the end of the experiment. The incorporation of [^{14}C]phenylalanine occurs during a much longer time than the incorporation of [^{14}C]glycine; it could be explained by the fact that [^{14}C]phenylalanine is not diluted with a locally synthesized amino acid, and perhaps by the low content of protein in the diet. The same discussion could be applied to myosin.

These results do not favour a conversion's taking place in the free amino acid pool. Other evidence in the same direction is given by our data on plasma and liver proteins, which show a very rapid equilibration of the specific activities of the different amino acids. Since this was not the case for haemoglobin and myosin, it is probable that contributions from the pool of hepatic amino acids is not determinant in our case.

Mechanism of the conversion. Our results bear some analogy to observations which have been made on collagen: in this tissue it was demonstrated that hydroxyproline arises only from proline, and hydroxylysine from lysine, free hydroxyproline and hydroxylysine being not or little incorporated (Stetten, 1949; Piez & Likins, 1957).

The mechanism of the conversion has given rise to several hypotheses which have not yet been demonstrated: (a) conversion of amino acids at the time of incorporation itself (Van Slyke & Sinex, 1958; Sinex, Van Slyke & Christman, 1959); (b) transformation of a precursor protein into collagen, involving the hydroxylation of the two amino acids (Stetten, 1949; Piez & Likins, 1957); (c) presence of hydroxyproline in a special form, 'bound' but not yet incorporated (Robertson, Hiwett & Herman, 1959; Green & Lowther, 1959). Hydroxyproline-containing peptides have been described which could represent the intermediate form (Chvapil & Cmuchaľova, 1960).

Analogous hypotheses could be made to interpret our results: (a) The conversion at the time of incorporation would involve the same requirements as before the incorporation, which in our case has been excluded. (b) The transformation of haemoglobin in the course of its aging would involve a change of the primary structure of haemoglobin with the age of the red blood cells. This hypothesis has been checked; we have estimated tyrosine in haemoglobin of the young and the old red cells fractionated by centrifuging (Pranker, 1958), and no change was found. (c) The temporary existence of amino acids in a special form which would not mix with the amino acid pool has to be discussed. With haemoglobin and myosin, these amino acids could occur not only at the time of their biogenesis, but also during the eventual turnover of these proteins. We have shown (Kruh *et al.* 1957, 1960)

that the unequal labelling of the haemoglobin after incorporation of ^{14}C from glycine or phenylalanine disappears in the course of the aging of the red cell.

This phenomenon can be interpreted by a three-step mechanism involving liberation of the amino acid from the protein, passage through a pool not mixed with the general pool, re-incorporation of the amino acid in the protein chain. These steps have to be discussed. A temporary liberation of an amino acid and its re-incorporation are one of the mechanisms assumed in the intramolecular turnover. The haemoglobin would then enter the general pattern of the turnover. But this turnover would be purely internal, within the red cell, which would take no part in the general metabolic turnover of the rest of the body, since there is no overall decay of the radioactivity of globin.

In the present work one more step is involved, i.e. the transformation of glycine into serine and phenylalanine into tyrosine. It should take place in the local pool before the re-incorporation.

SUMMARY

1. Rats given a low protein diet were injected with radioactive glycine and phenylalanine, and the specific activities of glycine, serine, phenylalanine and tyrosine were determined in purified globin and myosin at intervals of from 2 to 40 days after injection.

2. It was shown that conversion of glycine into serine and of phenylalanine into tyrosine occurs after the incorporation of the injected amino acids into haemoglobin and myosin.

3. The role of the general pool of amino acids of the organism is discussed and excluded. The mechanism of these conversions is discussed.

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Pyrophosphorolysis and Enzymic Synthesis of Cytidine Diphosphate Glycerol and Cytidine Diphosphate Ribitol

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Cytidine diphosphate glycerol and cytidine diphosphate ribitol were first isolated from ethanol extracts of *Lactobacillus arabinosus* (Baddiley & Mathias, 1954). The analyses and chemical syntheses of these two cytidine nucleotides showed that they were compounds of L- α -glycerol phosphate or D-ribitol 5-phosphate joined in pyrophosphate linkage to cytidine 5'-phosphate (Baddiley, Buchanan, Mathias & Sanderson, 1956c; Baddiley, Buchanan, Carss & Mathias, 1956a; Baddiley, Buchanan, Carss, Mathias & Sanderson, 1956b; Baddiley, Buchanan & Carss, 1957; Baddiley, Buchanan & Sanderson, 1958; Baddiley, Buchanan & Fawcette, 1959). More recently cytidine diphosphate glycerol has been detected in extracts of *L. plantarum* and *L. bulgaricus*, and cytidine diphosphate ribitol has been found in *L. plantarum*, *Bacillus subtilis* and (a trace) in penicillin-treated *Staphylococcus aureus* (Clark, Glover & Mathias, 1959). Strominger (1959) has found that *S. aureus* (Copenhagen) accumulated large quantities of cytidine diphosphate ribitol when treated with a preparation of gentian violet.

Nucleotide compounds of analogous structure, such as uridine diphosphate glucose (Munch-Petersen, Kalckar, Cutolo & Smith, 1953), guanosine diphosphate mannose (Munch-Petersen, 1955b) and cytidine diphosphate choline (Kennedy &

Weiss, 1956), are synthesized enzymically by reactions between the corresponding nucleoside triphosphates and the phosphate esters of the sugar or alcohol moiety. This type of reaction was discovered by Kornberg (1948, 1950) during studies on the biosynthesis of diphosphopyridine nucleotide and flavin-adenine dinucleotide. It seemed possible therefore that the biosynthesis of cytidine diphosphate glycerol and cytidine diphosphate ribitol might take place in a similar manner:

The present work is concerned with a search for pyrophosphorylases catalysing these reactions in cell-free extracts of *L. arabinosus* (the source of the nucleotides) and of a number of other micro-organisms. A preliminary account of some of this work has been published (Shaw, 1957).

EXPERIMENTAL

Materials

Nucleotides. Adenosine triphosphate (ATP), CTP, cytidine diphosphate (CDP), inosine triphosphate (ITP) and DPN were products of the Sigma Chemical Co. (St Louis, Mo., U.S.A.). Uridine triphosphate (UTP) was

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