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PREFACE

In the year that has elapsed since publication of the fourth volume of the *Review* there have been no changes in editorial or publication policy. In consequence, the need of a preface to the present volume might well be questioned were it not for the fact that such introductory paragraphs permit us to express the sense of appreciation that we feel to those whose labors and cordial suggestions are responsible for the volume.

If we may be permitted to speak for those who use the *Review*, more than a word of thanks must be conveyed to the contributors. Almost every field of biochemistry continues to receive extensive inquiry and the number of papers calling for careful appraisal steadily increases. Selection of the papers from which a review is to be woven, judicious weighing of the evidence presented, and synthesis of the material into a readable survey is a most difficult task. Perhaps the most unhappy feature is the necessity of leaving unmentioned a considerable number of excellent papers which temporarily must be placed aside because of the exigencies of space or because they pertain to subjects beyond the limits of the survey. The co-operation of the contributors has been so cordial and their attention to the exacting duties of a reviewer so complete that we wish most sincerely to endorse the sentiments of gratitude expressed by subscribers.

We continue to be indebted to the readers of the *Review* who, in increasing numbers, assist us with suggestions with respect to authorship, subjects appropriate for survey, and other matters which bear upon the quality and utility of the *Reviews*.

May we also express our gratitude to those who have been good enough to provide contributors with reprints of their published papers. In many instances, especially in the case of reports appearing in the less accessible journals, these reprints have been of the greatest assistance.

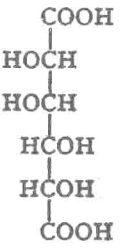
C. L. A.
D. R. H.
J. M. L.
C. L. A. S.

ERRATA

The following errors have been brought to the attention of the editor:

Volume I, page 413: *for* Thompson, *read* Thomson.
Page 426, line 11: *for* Cornell, *read* Corneli.
Page 427, line 5: *for* Cornell, *read* Corneli.

Volume II, page 38: Delete subscript 2 from the beta carbon atom in the graphic formula.
Page 44, first formula from left should read:

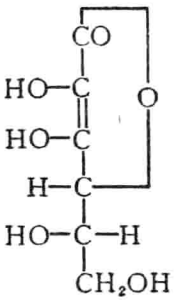


Page 59, seventh line from bottom: *for* 2,3,6-glucopyranose, *read* 2,3,6-trimethyl-glucopyranose.
Page 60, line 10: *for* 2,3,6-tetramethylglucose, *read* 2,3,6-trimethyl-glucose.
Page 74, tenth line from bottom: *for* prolin: gelatin, 0.9, *read* proline: gelatin, 9.0.
Page 101, twelfth line from bottom: *for* *dl*-methionine, *read* *dl*-Methionine.
Page 465, fifteenth line from bottom: *for* 5,5-dimethyl-cyclohex-andian-1,3, *read* 5,5-dimethyl-cyclohexanediol-1,3.
Page 528, line 9: *for* capronic, *read* caproic.

Volume III, page 127, line 31: *for* increase, *read* decrease.
Page 195, line 29: *for* histidine, *read* histamine.
Page 197, line 12: *for* Cystine, *read* Cysteine.

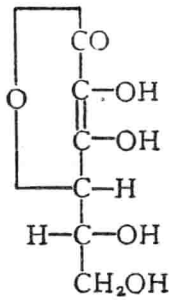
Volume IV, page 2, line 11: *omit* inversely.
Page 51, third line from bottom: *for* arginine, *read* arginase.
Page 100, Table II, line 2:
for Methyl alcohol....25.7 1183 1498,
read Ethyl alcohol....25.1 1156 1464.
Page 163, line 22: for *in vitro* as *in vivo;* read *in vivo* as *in vitro.*
Page 286, formula XIV: Add CH_3 group.

Page 349: The formulae for *l*- and *d*- and *iso*-ascorbic acid are better shown as follows:

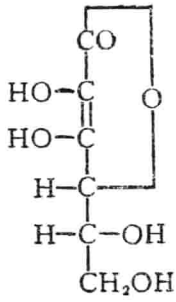


l-Ascorbic acid
Vitamin C

z

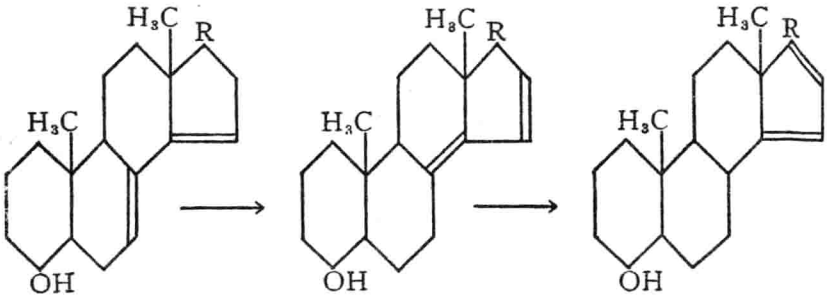


d-Ascorbic acid



d-Erythro-3-ketohexonic acid
"Isovitamin C"

Page 364: Rosenheim and King's suggested provisional formulae for tachysterol, intermediate product, and calciferol, should have been written:



Tachysterol

Calciferol

CONTENTS

BIOLOGICAL OXIDATIONS AND REDUCTIONS*

By D. E. Green[1] and D. Keilin

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In this review it is proposed to deal mainly with the mechanism of intracellular oxidation reactions, and with the properties of the components of cellular oxidation systems. Studies of the intermediary metabolism of animal tissue or bacterial cells will be considered here only in so far as they bear directly on the main problem.

Coenzymes

Coenzyme of hexose monophosphoric system.[2]—Warburg and his coworkers continued their investigation of the system which oxidises hexosemonophosphoric to phosphohexonic acid. This system which has been already described in the reviews of previous years is composed of a dehydrogenase (intermediary enzyme, or *Zwischenferment* of these authors), a coenzyme, hexosemonophosphoric acid, "yellow enzyme" (or flavoprotein compound), and molecular oxygen.

Warburg & Christian (1) have proceeded with the purification of the coenzyme, and from 250 liters of horse blood they have obtained 1 gm. of a highly purified product, 0.001 mg. of which can transfer 1 c.mm. of oxygen in one minute. The Q_{O_2} of the purified coenzyme is equal to 60,000, and 1 cc. of red blood corpuscles contains 0.012 mg. of this coenzyme.

Theorell (2, 3) by a cataphoretic method established that the coenzyme migrates anodically over the entire pH range, thus ruling out all possibility of a free amino group as in adenylic acid. This line of evidence was confirmed by the fact that nitrous acid does not inactivate the coenzyme. He further demonstrated the presence of two acidic groups with pK_1' equal to 1.8 to 1.9 and pK_2' equal to 6.2 to 6.3, respectively. From the fact that each acidic group involved two equivalents of hydrogen, he concluded that the coenzyme is a diphosphoric ester.

Warburg, Christian & Griese (4, 5) culminated their research of

* Received February 19, 1936.

[1] Beit Memorial Research Fellow.

[2] Cf. also this volume, pp. 31, 181. (Editor.)

the last four years with unquestionably the most fundamental contribution that has been made to the subject of coenzymes. They proved that the coenzyme is composed of one molecule of adenine, one of β-nicotinicamide, three of phosphoric acid, and two of pentose. Assuming six molecules of water of hydration, the molecular weight of the coenzyme should be 743. Molecular weight determination by the method of the depression of the freezing point of water yielded a value of 870; but considering the great uncertainty of this measurement, owing to the presence of many dissociable groups in the coenzyme molecule, the agreement is not unsatisfactory. The analysis figures agreed with the formula $C_{24}H_{28}O_{17}N_7P_3$. The experiments of Theorell (2, 3, 7) indicate that the third phosphoric acid group of the coenzyme is completely substituted and therefore does not titrate as an acid.

In presence of the dehydrogenase (*Zwischenferment*), hexosemonophosphate is oxidised to phosphohexonic acid while the coenzyme is simultaneously reduced. One molecule of hydrogen is transferred from the substrate to the coenzyme in presence of the enzyme. This reduction of the coenzyme can also be brought about by hydrosulphite ($Na_2S_2O_4$). Reduced coenzyme, formed either by enzymic reduction or by reduction with hydrosulphite, is reversibly oxidised by the yellow enzyme (flavoprotein) and the original coenzyme is regenerated. This reversible oxidation and reduction is the key to the catalytic functioning of the coenzyme. However, if the coenzyme is reduced by platinum and hydrogen in a slightly alkaline solution, one molecule of coenzyme takes up three molecules of hydrogen and forms a compound which can no longer be reoxidised by the yellow enzyme. In other words, the reduction with nascent hydrogen is an overreduction and is not reversible, and the catalytic activity of the coenzyme is therefore destroyed.

The coenzyme has no absorption bands in the visible region of the spectrum but shows a broad band at 260 mμ in the ultraviolet, which can easily be explained on the basis of the summation of the individual absorptions of both the adenine and β-nicotinicamide moieties of the molecule (the –C=N– groups). On reversible reduction, this band remains unchanged and a new band at 345 mμ appears. On irreversible reduction, the band at 260 mμ fades, and the absorption that remains is due only to the adenine moiety. From this evidence, Warburg *et al.* conclude that by irreversible reduction the pyridine ring of β-nicotinicamide is transformed into a piperidine ring. Piperidine

does not absorb appreciably at 260 mμ thus explaining the fading of this band on over-reduction. They have also found that if the coenzyme is previously reduced by the hexosemonophosphoric system and then treated with platinum and hydrogen, it takes up only two molecules of hydrogen instead of three in the latter reaction. These facts show that in the biological reduction of coenzyme, it is the pyridine ring only which takes up a molecule of hydrogen.

The interpretation of the spectroscopic data received confirmation from a similar study of the inner methyl betaine of nicotinic acid (trigonelline). On reversible reduction with hydrosulphite, this substance shows a new band at 345 mμ, while the original absorption remains unchanged. Like the partially reduced coenzyme, reduced trigonelline is also unstable in acid solution.

The mechanism which Warburg and his coworkers propose for the reaction between oxygen and the substrate is one involving (*a*) the transfer of two atoms of hydrogen from the substrate to the coenzyme, (*b*) the transfer of two atoms of hydrogen from the reduced coenzyme to the yellow enzyme, and (*c*) the transfer of two atoms of hydrogen from the reduced yellow enzyme directly to oxygen with production of hydrogen peroxide. The dehydrogenase is involved in step (*a*) only. A pyridine and an isoalloxazine ring provide the chemical pathway for the transference of hydrogen from hexosemonophosphate to oxygen.

The reversible reduction of the coenzyme either enzymatically or by hydrosulphite has been determined manometrically in the presence of bicarbonate and 5 per cent carbon dioxide. The oxidation products of sodium hydrosulphite ($Na_2S_2O_4$) or of hexosemonophosphate being acids, one molecule of evolved carbon dioxide indicates the transference of one molecule of hydrogen from the substrate to the coenzyme. Having established that the hydrogen capacity of 1 mg. of pure coenzyme is 30 c.mm., the hydrogen capacity per mg. of any preparation divided by 30 gives directly the measure of purity of the preparation with respect to coenzyme. Similarly from the known absorption coefficient of pure reduced coferment at 345 mμ, the concentration of coenzyme in any preparation can be determined spectrophotometrically after addition of the reducing agent.

Warburg, Christian & Griese, in their preliminary communication (4), have already advanced the view that the function of their *Zwischenferment* is that of a protein carrier for the coenzyme. This view was more recently developed by Negelein & Haas (6). By

studying the kinetics of the reduction of the coenzyme, varying in turn the concentration of coenzyme, enzyme, and substrate, they arrived at the conclusion that the enzyme acts only as a colloidal carrier and has no catalytic activity apart from the coenzyme. The enzyme and coenzyme combine reversibly to form the catalytically active complex which is the "hydrogen-transporting enzyme."

$$\text{Coenzyme} + \underset{\text{(Dehydrogenase)}}{\textit{Zwischenferment}} \rightleftarrows \text{Hydrogen-transporting Enzyme}$$

They estimate that when the concentration of coenzyme is 10^{-5} *M* half the active complex is dissociated into coenzyme and *Zwischenferment*. One molecule of active complex in presence of hexosemonophosphoric acid can hydrogenate at least 3400 molecules of coenzyme per minute. The relationship between the coenzyme and enzyme is, according to these authors, of the same nature as the relationship between the flavin and protein in yellow enzyme or even globin and heme in hemoglobin. These results are however not in agreement with those obtained by Theorell (7) who studied the migration of hexosemonophosphate, coenzyme, and dehydrogenase in an electric field. All these components were negatively charged and migrated anodically. From an analysis of the rate of migration of mixtures of the various components, he concluded that the coenzyme formed a compound with the substrate as did the enzyme. However, there was no indication of any compound formation between the enzyme and the coenzyme.

In the theory of Warburg and his coworkers, neither the formation of the compound between the enzyme and substrate nor the activation of the latter are considered.

Cozymase.[8]—In addition to the coenzyme of Warburg & Christian there have been several coenzymes described and isolated from animal and yeast cells which are required for the complete functioning of dehydrogenase systems. Outstanding in importance among these is the Euler & Myrbäck cozymase of yeast. Warburg & Christian (1) proved conclusively that their coenzyme could not replace cozymase as the activator of alcoholic fermentation. Thus it is clear that they are not identical although their chemical composition and properties, as we shall see, have much in common.

Euler, Albers & Schlenk (8) succeeded in purifying cozymase

[8] Cf. also this volume, pp. 30, 190. (EDITOR.)

from[4] ACo 110,000 to ACo 350,000 to 400,000 by precipitation as the cuprous salt. On the basis of Myrbäck's & Euler's (10) determination by the method of diffusion, Euler *et al.* (8) assumed the molecular weight to be about 394, and found the analysis figures to agree with the formula $C_{12}H_{19}O_9N_4P$. This value is half that assigned to the coenzyme from red blood corpuscles. In a later note, Euler, Albers & Schlenk (11) modified their empirical formula to $C_{24}H_{35}O_{18}N_8P_2$ which corresponds to a molecular weight of 785. Hydrolysis in acid solution yielded adenine to the extent of 19.5 per cent of the total weight of coenzyme. The amide of β-nicotinic acid was also isolated as the picrolonate but to the extent of only 47 per cent of the theoretical quantity expected on the basis of the dinucleotide structure of cozymase. The essential chemical differences between the two coenzymes seem therefore to consist in cozymase having one phosphoric acid group less than the coenzyme of blood and having less than one molecule of nicotinicamide per molecule of adenine contrasted to their 1 : 1 ratio in blood coenzyme.

Myrbäck & Örtenblad (12 to 17) published a series of papers dealing with the chemistry of cozymase. Since the ACo's of their preparations were substantially less than 200,000 units, the purity of the coenzyme preparations under study must have been less than 50 per cent. Although the chemical analyses of these preparations are still inadequate, Myrbäck & Örtenblad (13, 17) succeeded nevertheless in obtaining some very interesting information on the chemical properties of cozymase. A strongly reducing group in the cozymase molecule could not be ascribed either to the pentose or the adenine moieties. They concluded from various lines of evidence that this reducing power was due to some nitrogenous base, as yet unknown. The isolation of β-nicotinicamide by Euler *et al.* was the logical development of Myrbäck's observations and of the work of Warburg *et al.* Myrbäck found further that reduction with nascent hydrogen completely inactivated cozymase and that this inactivation was correlated with a significant change in the reducing properties of cozymase. In the light of the results obtained by Warburg *et al.* it appears that over-reduction of cozymase by nascent hydrogen explains this inactivation.

Other coenzymes.—An extensive literature dealing with the interchangeability of coenzymes for various biological processes has grown

[4] The unit of cozymase activity of Myrbäck (9): ACo = cc. of carbon dioxide per gm. (dry weight) per hour.

up in the last few years. Since most of the investigators worked with mixtures of coenzymes, a certain number of discrepancies was inevitable.

It was found that the coenzyme of Warburg & Christian can act catalytically with the hexosemonophosphate system of yeast and blood [Warburg & Christian (18)], the lactic and malic dehydrogenases of heart [Wagner-Jauregg *et al.* (20)], the glucose dehydrogenase of liver [Euler *et al.* (19)], and the citric dehydrogenase of cucumber seeds [Wagner-Jauregg & Rauen (21)]. The cozymase of Euler & Myrbäck works with the alcohol dehydrogenase of yeast [Euler & Adler (23)], lactic dehydrogenase of heart [Banga & Szent-Györgyi (24)], malic dehydrogenase of heart [Ogston & Green (22)], glucose dehydrogenase of liver and citric dehydrogenase of cucumber seed [Andersson (26)].

The coenzyme of heart lactic-acid dehydrogenase (27) has been assumed by Banga & Szent-Györgyi (24) to be identical with cozymase on the basis of their interchangeability. This conclusion, however, may be premature in view of the description by Banga, Szent-Györgyi & Vargha (28) of chemical properties of the heart lactic-acid coenzyme which do not correspond to those of cozymase. The non-identity of the two coenzymes is maintained also by Birch & Mann (29). On the other hand, the glucose coenzyme of Harrison has been shown by Andersson (26) to be identical with cozymase.

It may be mentioned here that according to Adler & Michaelis (30) and Ogston & Green (22), lactic dehydrogenase of yeast, for the reaction with methylene blue, does not require the co-operation of either coenzyme or yellow enzyme.

It is noteworthy that according to Euler *et al.* (19) adenylpyrophosphate has no demonstrable activity as a coenzyme of oxidative processes. There have been reports from time to time of positive results obtained with this substance but these results are probably due to contamination of adenylpyrophosphate with cozymase or other coenzymes. The rôle of adenylpyrophosphate, as shown by Parnas and his school, is that of phosphate transfer exclusively.

Euler & Adler (31) and Ogston & Green (22) independently showed that cozymase was inactive with respect to the hexosemonophosphate-dehydrogenase system in contradiction to the earlier positive finding of Euler & Adler (32). The former group of workers attributed the discrepancy in their results to an impurity in their original preparations. The impurity in conjunction with cozymase

makes for catalytic activity. They concluded that this impurity[5] is not identical with the Warburg coenzyme. Crude preparations of hexosemonophosphate dehydrogenase of yeast are rich in this impurity, which is dialysable and thermolabile. This factor is not required for the catalytic activity of cozymase in the alcohol-dehydrogenase system. Birch & Mann (29) reported a similar separation of the lactic coenzyme into two factors.

Euler & Vestin (33) and Euler & Günther (25) pointed out that thermo-inactivated cozymase was still active as the coenzyme of lactic acid formation by muscle extract in presence of hexosediphosphate, although it was completely inactive in alcoholic fermentation by yeast. They concluded that there were two functional groups in cozymase: one concerned with oxidative and fermentation processes, the other with phosphorylation and lactic acid production.

Euler & Adler (34) found that after adding adenylpyrophosphate to the hexosemonophosphate-dehydrogenase system of yeast (composed of yeast dehydrogenase, coenzyme, and yellow enzyme) both glucose and fructose could be oxidised, although in absence of the nucleotide no oxidation of unphosphorylated hexose ensues. They showed that in their preparation of the dehydrogenase there was also present a phosphorylating enzyme capable of catalysing the transference of phosphate from adenylpyrophosphate to glucose or fructose. The phosphorylated sugar thus formed is oxidised in the usual way by the dehydrogenase in presence of the coenzyme and yellow enzyme. The phosphorylating enzyme (heterophosphatese) can be separated from the dehydrogenase and its action is not dependent upon a simultaneous oxidation process. The rôles of adenylpyrophosphate and coenzyme are therefore not interchangeable. While the former acts only as a substrate for phosphorylation of glucose, the latter is concerned with the oxidation of phosphorylated hexose thus formed. It is interesting to note that in this reaction of phosphorylation, adenylpyrophosphate cannot be replaced by either muscle- or yeast-adenylic acid or creatine or even phosphocreatine.

Runnström & Michaelis (35) report synthesis of organic phosphate as a consequence of the oxidation of hexosemonophosphate by blood hemolysate (containing the dehydrogenase + coenzyme) taking place in the presence of yeast cozymase and methylene blue. With-

[5] Note added March 31. Euler & Adler have rejected their earlier evidence and consider the impurity to be the Warburg coenzyme [*Z. physiol. Chem.*, **238**, 233 (1936)].

out yeast cozymase, no appreciable synthesis takes place. Pyocyanine can replace cozymase and methylene blue. Synthesis does not occur without oxidation but the reverse takes place usually in absence of yeast cozymase.

Schäffner, Bauer & Berl (36) find that the phosphorylation of hexose by yeast phosphatese requires the presence of the hexosephosphate-dehydrogenase system.

Flavins[6]

Karrer, Schöpp & Benz (37) and Kuhn, Reinemund, Weygand & Ströbele (38) independently and practically at the same time announced the synthesis of 6,7-dimethyl-9-(*d*,1′-ribityl) isoalloxazine which was shown to be identical with lactoflavin in all respects. Theorell (39) purified the prosthetic group of the Warburg yellow enzyme and isolated a phosphoric compound of the composition $C_{17}H_{21}O_9N_4P$. In contrast to lactoflavin, the prosthetic flavin contains one atom of phosphorus. Kuhn & Rudy (40) phosphorylated lactoflavin with $POCl_3$ and obtained a derivative with one atom of phosphorus. This compound, however, was found not to be identical with Theorell's flavinphosphate as shown by the inability of the synthetic flavinphosphate to combine with the carrier protein of the yellow pigment. Theorell, Karrer, Schöpp & Frei (41) prepared flavinphosphate from liver. Their preparation was not analytically pure but they were able to demonstrate a partial synthesis of yellow enzyme from the flavinphosphate and the specific protein. Rudy (42) recently announced the phosphorylation of lactoflavin by intestinal phosphatese in presence of inorganic phosphate. After several hours incubation with the enzyme system, the dialysed flavin migrated anodically, i.e., in reverse direction to lactoflavin.

According to Pett (43) bottom yeast grown in presence of cyanide, cysteine, or pyridine possessed a flavin content much higher than normal. This was correlated with a decrease in the Q_{O_2} although the $Q_{CO_2}^{N_2}$ remained normal. A certain amount of phosphate must be present in the medium for normal development of flavin.

Ogston & Green (22) demonstrated the inactivity of lactoflavin as a hydrogen carrier for isolated dehydrogenase systems.

Yellow enzyme of Warburg & Christian (flavoprotein compound).—Theorell (44), after a long series of purifications, succeeded in crystallising the yellow enzyme, the molecular weight of which he

[6] Cf. also this volume, pp. 33, 189, 359. (Editor.)

estimates at 70,000, on the basis of 0.6 per cent flavinphosphate content in the purest preparation. When a solution of yellow enzyme is dialysed against 0.02 *N* HCl for seventy-two hours, the prosthetic group is split off and diffuses into the outside fluid. The colourless inside solution is then dialysed against distilled water to remove hydrochloric acid, and after centrifugation a clear solution of the protein component of the yellow enzyme is obtained. When flavinphosphate, prepared by methyl alcohol extraction of the yellow enzyme, is mixed with the protein separated from the enzyme, they rapidly combine, resynthesising the original compound. On the other hand, the non-phosphorylated lactoflavin does not form a compound with the protein of the yellow enzyme.

Adler & Euler (45) and Ogston & Green (22) showed that yellow enzyme can function as an efficient oxygen carrier for the isolated glucose-dehydrogenase system of liver. The latter investigators and Hahn, Niemer & Freytag (46) found similar catalytic activity of the yellow enzyme with the hexosediphosphate systems of blood and of yeast. Wagner-Jauregg & Rauen (21) reported that yellow pigment is also active in the citric dehydrogenase system of plant seeds.

Meyerhof & Schulz (47) have found that in the hexosemonophosphate system composed of the dehydrogenase, coenzyme, hexosemonophosphoric acid, and the yellow enzyme, oxygen can be replaced by NO which becomes thus reduced to N_2O. It is interesting to note that the rates of reduction of NO and of oxygen are practically the same.

Cytochrome[7]

Theorell (48) extracted cytochrome-*c* from horse and ox heart, and after purification by dialysis, and by acetone and ammonium sulphate fractional precipitation obtained a product with 0.17 to 0.25 per cent of iron. By cataphoretic separation of impurities, the percentage of iron rose to 0.31 to 0.33. Further treatment by precipitation of the picrolonate yielded the purest preparation with an iron content of 0.34 per cent. One gram of cytochrome-*c* of this purity was isolated from 100 kg. of heart. Cytochrome-*c* was found to be strongly basic with an isoelectric point of 9.7 according to cataphoretic data. On treatment with a platinum catalyst and hydrogen, cytochrome-*c* is reduced—one atom of hydrogen being absorbed per atom of iron in agreement with the earlier results of Hill & Keilin (49)

[7] Cf. also this volume, p. 476. (Editor.)

and Green (50). Zeile (51) purified cytochrome-*c* of yeast by adsorption on and elution from kaolin. The hematin content of his purest preparation was 3.5 per cent. The isoelectric point of yeast cytochrome-*c* is 8.2. Yakushiji (52) extracted cytochrome-*c* from higher plants and algae and found the extracts to have properties similar to the cytochrome-*c* solutions from yeast. Roche & Bénévent (53) redetermined the absolute absorption spectrum of cytochrome-*c* of yeast, and confirmed the results obtained by Dixon, Hill & Keilin (54). They repeated also the experiments of Keilin (55) and Zeile (56) who showed that protohematin of blood on repeated oxidations and reductions gives rise to a hematin which is very similar to that of cytochrome-*c*. The hematin-*c* thus obtained differs according to Roche & Bénévent from all the other hematin compounds by its inability to combine with native globin to form methemoglobin. This seems to show that the elements of heme which unite with globin to form hemoglobin are not identical with those which combine with other nitrogenous substances to form parahematin and hemochromogen.

Ogston & Green (22, 57) tested the ability of cytochrome-*c*, glutathione, flavin, and yellow enzyme to catalyse the reaction of eleven dehydrogenase or oxidase systems with molecular oxygen. While yellow enzyme can act as a carrier with several dehydrogenases such as hexosemonophosphoric, hexosediphosphoric, glucose, and malic, cytochrome-*c* from yeast has catalytic activity only with the succinic dehydrogenase of animal tissues and the lactic dehydrogenase of yeast. The mechanism of this catalysis involves the collaboration of two enzyme complexes: the dehydrogenase-substrate system which reduces oxidised cytochrome and the indophenol oxidase-oxygen system which oxidises reduced cytochrome.

Haas (58) calculated from spectroscopic data the percentage of the total respiration of bakers' yeast which proceeds through cytochrome. The observed rate of reduction of cytochrome was found to account for the entire respiration within the limits of experimental error. Warburg & Christian (1) similarly calculated from the turnover of the yellow enzyme that only 1/160 of the respiration of bakers' yeast can be assumed to involve the yellow enzyme. Furthermore, systems which react with yellow enzyme are insensitive to cyanide and carbon monoxide and produce one mol of hydrogen peroxide for each mol of reduced yellow enzyme that autoxidises. Most living cells, however, are sensitive to both cyanide and carbon monoxide. The fact that *in vitro* only two out of several dehydrogenases

react with cytochrome-*c* can mean only that some essential factors or components are still missing in the reconstructed system. A striking illustration of the discrepancy between *in vitro* and *in vivo* results is given by Ogston & Green in their experiments on bottom yeast. This organism contains a relatively large quantity of yellow enzyme. Yet, although equipped with dehydrogenase systems which *in vitro* can react with oxygen via yellow enzyme, its respiration is negligible in the presence of the appropriate substrates with or without added coenzyme. The addition of pyocyanine and coenzyme produces *in vivo* an enormous increase in respiration despite the fact that pyocyanine is only one-third as active as yellow enzyme *in vitro* [Green, Stickland & Tarr (59)].

Glutathione

Meldrum & Tarr (60) found that the hexosemonophosphate system of yeast or mammalian red blood corpuscles can reduce glutathione very rapidly. Hexosediphosphate and phosphohexonic acid can replace hexosemonophosphate in the yeast system but the rate of reduction of glutathione then becomes much slower. The limiting factor in the reaction of the hexosemonophosphate-glutathione system with oxygen is the speed of autoxidation of the reduced tripeptide. The experiments of Meldrum & Tarr provide a simple explanation of the difference between the two coferments which Warburg & Christian (1) isolated from red blood corpuscles. Coferment II, unlike I, does not require the addition of carrier in order to catalyse the reaction between hexosemonophosphate and oxygen. Apparently coferment II is rich in glutathione, thereby the necessity for additional carrier is obviated. Negelein in unpublished experiments quoted by Warburg & Christian (122) independently discovered the presence of glutathione in coferment II. Wagner-Jauregg & Möller (61) reported that reduced glutathione increases the rate of reduction of methylene blue by the alcohol-dehydrogenase system of yeast. They explain the effect as one of heavy metal inactivation. Kubowitz (62) prepared ferroglutathione and demonstrated the light sensitivity of the carbon monoxide compound formed.

Ghosh & Ganguli (63) have studied the reversibility of the glutathione system.

The Rôle of Fumaric Acid in Respiration

Gözsy & Szent-Györgyi (64), already in 1934, suggested that in cellular respiration the succinic-fumaric system may act as a link

between the substances metabolised and the oxidase-cytochrome system. This hypothesis was based mainly on the observations that the respiration of minced pigeon-breast muscle suspended in phosphate buffer solution is strongly inhibited by malonic and maleic acids. More recently, Szent-Györgyi and his coworkers (65 to 71) have revised this hypothesis and have modified it considerably; it is not the succinic-fumaric, but the fumaric-oxalacetic system which makes the link between the activated molecules of substrate and the oxidase-cytochrome system.

In this scheme the substrate molecules, activated by their corresponding dehydrogenases, react with oxalacetic acid and become oxidised while oxalacetic acid becomes reduced to fumaric acid. The latter, activated by the fumaric-dehydrogenase system, is oxidised by reacting with the oxidase-cytochrome system, not directly but through the medium of an undetermined thermolabile intermediary substance.

According to Szent-Györgyi and his coworkers the following considerations can be brought forward in support of this theory:

a) The initial rate of respiration of the pigeon-breast muscle (pulp or slices) suspended in phosphate buffer solution rapidly falls off, but can be kept up for a long period by the addition of a little fumarate or oxalacetate to the medium. The addition of this substance does not increase the initial rate of respiration but only stabilises it.

b) Oxalacetic acid added to muscle pulp in the absence of oxygen undergoes rapid reduction, which, they calculate, can account for the total transfer of hydrogen in normally respiring tissue.

c) The respiration of muscle tissue is strongly inhibited by maleic acid which affects presumably only the fumaric-oxalacetic system.

d) The addition of arsenite, which has a much greater inhibitory effect on various dehydrogenases than on the dehydrogenase oxidising fumaric to oxalacetic acid, enabled them to detect the presence of oxalacetic acid in muscle tissue kept aërobically and also to reveal the existence of fumaric dehydrogenase by the Thunberg method.

e) The oxygen uptake of muscle tissue in phosphate buffer solution is strongly inhibited by malonic acid, and this inhibition is completely abolished by the addition of fumaric acid. This observation, which was in contradiction with their previous hypothesis, is in agreement with their revised views and also connects their system with the powerful succinic dehydrogenase. They assume that in an actively respiring tissue the oxalacetic acid may undergo over-reduction to succinic acid, which is very rapidly re-oxidised back to fumaric