the molecular volume by the transformation increases from 45.37 Å³ to 47.12 Å³. The rotating molecules also explain the rapid fall of the Röntgen-ray reflections with increasing deflection-angle. That molecular rotation can occur in the solid state is evident from the two modifications of hydrogen, and the problem has been theoretically treated by Paceling. In this connection, it is of interest to mention that the oxygen modification, stable above −229.5, was recently found by us to have a cubical lattice with rotating molecules. On the basis of our interpretation of the luminescence, we immediately see the reason why the β-modification has lost its phosphorescent power.

The rotational motions of the molecular elements of the lattice will disturb the metastable states just as in the gaseous system before the forbidden transitions can take place.

Reasons are given for the view that the excitation consists in a kind of dissociation process and that recombination takes place through certain metastable states. The phosphorescence, at any rate in our case, should be closely related to chemiluminescence, the main difference being that in the case of phosphorescence the reacting chemical substances are first to be formed through the excitation process.

L. VEGARD

COUPLED REACTIONS IN BIOLOGICAL SYSTEMS

THE following is a summary of some preliminary investigations of two coupled reactions in biological systems: (1) The reduction of pyruvate to lactate by means of the energy of the anaerobic oxidation of formate to bicarbonate, and (2) the reduction of fumarate to succinate through the anaerobic oxidation of lactate to pyruvate. Toluene treated *B. coli* served as enzyme system for both reactions.

It was found in both instances that an intermediate substance was necessary which could be reduced at the locus where the one metabolite was oxidized, and reoxidized where the other was reduced. The intermediate substances were methylene violet for the lactate-pyruvate-formate-bicarbonate system, and methylene blue for the succinate-fumarate-lactate-pyruvate system. Without these mediators no reaction occurred.

These findings support the hypothesis of active centers proposed by Quastel.[1] They indicate that "half reactions" can not occur, and therefore the necessity for mediators, or carriers of energy from the point where energy is liberated to the point where it is used, when the energy-liberating and energy-absorbing mechanisms are separate; *i.e.*, that in the toluene treated *B. coli* there is no mechanism corresponding to metallic conduction. They show also that toluene treated *B. coli* contain no mediators capable of serving in the two systems studied.

Further, these experiments suggest that when oxygen is used *in vivo* the centers at which oxygen is bound must be intimately associated with the dehydrogenase mechanisms, or that one or more mediators similar in their function to reversible dyes intervene between the oxygen binding and the dehydrogenase mechanisms. These alternatives are not mutually exclusive. They may co-exist and even supplement each other, as possibly, for example, in the case of the non-iron-containing respiratory ferment system described by Warburg and Christian.[2]

HERMANN F. SCHOTT
HENRY BORSOOK
CALIFORNIA INSTITUTE OF TECHNOLOGY

SOME ASPECTS OF INTERMEDIARY PROTEIN METABOLISM

THE exogenous origin of creatine was first demonstrated by Levene and Kristeller in 1909 in their protein feeding experiments with cases of muscular dystrophy. In 1929, we reported[1] that glycine fed to patients with muscular dystrophy produced a marked rise in creatine excretion (confirmed by Thomas *et al.*,[2] and others), whereas glutamic acid and cystine had no effect. Studies with a number of other amino acids and various other substances were also reported. The effect of the removal of glycine and of glutamic acid from the metabolic mixture through the feeding of benzoic acid and phenylacetic acid, respectively, was investigated. It was found that the former produced a marked drop in creatine, while the latter was without effect. We are inclined to infer from our experiments that the feeding of brombenzene, which removes cysteine from the metabolic mixture, would be without effect on creatine excretion. Owing to the possible toxicity of the substance we intend to carry out suitable animal experiments instead. The chart shows only the essential results of a few representative experiments.[3]

Hippuric acid, phenylacetyl glutamine and bromphenyl mercapturic acid formation: The finding that the feeding of benzoic acid and phenyl acetic acid in proper amounts may have no effect upon the total nitrogen catabolism, although there are appreciable amounts of glycine and glutamic acid, respectively, lost to the body as a result of "Abfang" processes,

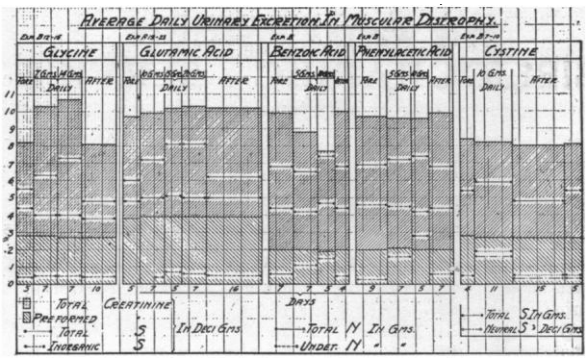
[1] J. H. Quastel, *Biochem. Jour.*, 20: 166, 1926.

[2] O. Warburg and W. Christian, *Biochem. Zeits.*, 258: 496, 1933.

[1] *Am. Jour. Physiol.* 90, 296.

[2] *Z. physiol. Chem.* 214, 121.

[3] The significance of our detailed nitrogen and sulfur studies can not be discussed here, due to lack of space.



we believe, would indicate that these amino acids are originally present in the metabolic mixture in appreciable amounts and not especially synthesized for detoxification. This view is substantiated by our findings that creatine is markedly reduced by the benzoic acid and uninfluenced by the phenyl acetic acid and also by our observations regarding the respective influence of glycine, glutamic acid and cystine upon the excretion of creatine.

The rôle of glutathione in intermediary protein metabolism: It is a surprising fact that the only three amino acids—glycine, glutamic acid and cysteine —which seem to be available for processes of detoxification in the human are the same as those which form glutathione.[4] If glutathione supplies the amino acids for these processes of detoxification, then the unaltered level of protein catabolism accompanying such processes would seem to indicate that an appreciable part of normal protein catabolism goes *via* glutathione. It is possible that this view may be further supported by the finding that methionine replaces cystine in cystine deficient diets (Jackson and Block) and by the observation regarding the influence of methionine on the detoxification of brombenzene in such diets (White and Lewis).

One may conceive that the substances, which are being detoxified, first combine with the glutathione, thereby rendering it more unstable, as a result of which the detoxified compound splits off in combination with part of the original glutathione molecule. This change in stability would be in keeping with our findings regarding factors which influence the lability of the glutathione and cystine molecules.[5]

E.g., phenylacetic acid would combine with the α-amino group of the glutamic acid radical of glutathione, following which the side chain splits off completely at the α-carbon of the cysteine liberating phenylacetylglutamine.

Shiple and Sherwin have called attention to the obviously close relationship between phenylacetylglutamine excreted by man and phenylacetylornithine excreted by birds. Ornithine in this latter compound may be conceived as derived from glutathione by a process in which the distant carbonyl group of the glutamic acid radical is reduced to form a nor-valine radical, which if split off together with the α-amino group of the cysteine would yield ornithine. Such mechanisms may have some bearing on uric acid and pyrrole formation. The possibility of glutathione being a source of ornithine is of interest in connection with the work of Krebs on urea formation and the reported activation of arginase by glutathione. The glutamic acid linkage in glutathione, which linkage is not easily split by enzymes, has long been considered as unusual. The reactivity of the distant carbonyl group of the glutamic acid radical in glutathione hardly has been investigated.

Bromphenyl mercapturic acid referred to above contains an acetyl group, which may be a remnant of the glutamic acid radical of glutathione, rather than the result of acetylation.

Creatine formation: Our experiments indicate that the guanidine group of creatine is synthetic in origin and that glycine is involved in creatine formation. Since glutathione is now known to contain glycine, its possible relation to creatine formation is obvious.

Creatinine formation: Creatinester hydrochloride and related compounds spontaneously form their respective anhydrides upon neutralization, the free esters apparently being quite unstable (unpublished experiments by Failey and Brand). This behavior of creatinester might suggest that in the animal organism creatinine is formed from a creatine compound in which the carboxyl group of creatine is not free but linked up as an ester, thioxy ester or amide.

Our finding in cystinuria[6] regarding an unusual creatinine metabolism accompanying this disturbance in sulfur metabolism is of interest in this connection. Studies regarding the therapeutic and metabolic effects of glutathione and of mixtures of its constituent amino acids in myopathies are being planned.

We shall consider more fully some of the conceptions of protein metabolism outlined above and their implications in a more detailed publication of our experiments.

This work was aided in part by a grant from the Chemical Foundation.

ERWIN BRAND
MEYER M. HARRIS

NEW YORK STATE PSYCHIATRIC INSTITUTE AND HOSPITAL

[4] This view has been expressed previously by others (Waelsch, H., *Arch. Exp. Path.* 156, 356; Power, F. W., Washington Meeting, Am. Chem. Soc. (1933)).

[5] *Jour. Biol. Chem.*, 70, 381.

[6] *Jour. Biol. Chem.*, 86, 315.