phorylation sites are involved, one of which is affected, whereas the other one remains unaffected. When the rate of light-induced phosphorylation is stimulated several fold by phenazine methosulfate, only a small part of the phosphorylation, which is about equivalent to the sensitive part of the unstimulated system, is inhibited by valinomycin. This supports the conclusion that one of two sites, the "valinomycin-insensitive" one, is activated by addition of phenazine methosulfate. "Cyclic" light-induced phosphorylation in spinach chloroplasts remains unaffected by valinomycin, which may indicate that only a site corresponding to the "valinomycin-insensitive" site in bacterial chromatophores is participating in light-induced phosphorylation of the isolated spinach chloroplasts.

(3) When a preparation of bacterial chromatophores has been aged at 55–57°C, the initially very low stimulation obtained by menadione increases with the aging time, up to 15–25 minutes of preaging, after which a very rapid decrease occurs. In contrast to the stimulation obtained by phenazine methosulfate, that obtained by menadione is very sensitive to low concentrations of the inhibitors antimycin A and 2-*n*-heptyl-4-hydroxyquinoline-N-oxide. Thus a difference has been found between the modes of action of the two stimulatory agents on light-induced phosphorylation.

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# Glycolysis in Human Erythrocytes Studied with $^{14}C$-labelled Glucose

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The energy required for the maintenance of viability of the erythrocytes is obtained through breakdown of glucose mainly to lactate. Some hemolytic anemias and the aging of erythrocytes *in vitro* or *in vivo* have been shown to be accompanied by disorders in the glycolytic system [1-4].

In order to study these disorders more closely a test system has been worked out. The erythrocytes are washed at 4°C and finally suspended in an inorganic buffered salt solution with the following composition: NaCl 108 mM; KCl 3.9 mM; $MgCl_2$ 5 mM; Na-phosphate (pH 7.4) 20 mM. 2 ml of the suspension (holding 15 % hemoglobin) are incubated at 37° in the two media described in Table 1. After 60 min the glycolysis is stopped by adding perchloric acid. Different perchloric acid soluble substances are then separated using stepwise elution through a Dowex 1 column according to Bartlett [4]. Methylene blue stimulates breakdown along the pentose phosphate shunt [5] and the $CO_2$ formed during incubation is absorbed to NaOH moistened filter paper strips in the center well of the Erlenmeyer

*Table 1.* Composition of media.

| | − methylene blue ml | + methylene blue ml |
|---|---|---|
| Erythrocyte suspension | 2.0 | 2.0 |
| Buffered salt solution | 0.6 | 0.6 |
| 0.05 % methylene blue | — | 0.2 |
| Distilled water | 0.2 | — |
| 0.15 M $^{14}C$-glucose (uniformly labelled, containing $1.3 \cdot 10^6$ cpm). | 0.2 | 0.2 |
| Total volume | 3.0 | 3.0 |

*Table 2.*

| | − methylene blue | + methylene blue |
|---|---|---|
| ATP, μmoles per incubation flask | 0.38 | 0.45 |
| 2,3-diP-glycerate, μmoles per incubation flask | 2.8 | 2.9 |
| % radioactivity in $CO_2$ | — | 3.5 |
| % » » 2,3-diP-glycerate | 2.7 | 4.8 |
| % » » lactate | 1.9 | 2.9 |

flask used for the incubation. Some parameters studied are listed in Table 2 together with the mean values of three different blood samples. Some examples of application will be given.

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## Dilatometry in the Study of Acyl Shift in Peptides and Proteins

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The dilatometer technique as described by Linderstrøm-Lang [1] has been introduced in our studies on the formic acid induced reversible inactivation of protein enzymes [2-4] in order to elucidate the mechanism of the reactivation process.

From our previous studies three reactions were thought possible in this process, namely 1. an O,N-acyl shift, 2. deformylation of the formylated amino groups, or 3. deformylation of the formylated hydroxyl groups of the hydroxyamino acid residues.

Therefore the volume changes accompanying some proton transfers as well as the O,N-acyl shift in some model substances have been determined in addition to those accompanying the reactivation of formic acid inactivated protein enzymes.

The volume change accompanying the reactivation of ribonuclease when performed in phosphate buffer, *i. e.* 15.3 ml per equivalent base consumed, is in good agreement with that accompanying the proton transfers in the neutralisation of formic acid with secondary phosphate ions, *i. e.* 15.7 ml. In contrast, the value found for an O,N-acyl shift is 21.1 ml. Thus the data strongly suggest that the reactivation is based on a deformylation process.

Dilatometer experiments on formic acid treated glycyl-DL-serine as well as glycyl-O-formyl-DL-serine further suggest that the deformylating process is a splitting of the formamid bond in the O-acyl formed structure.

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