

taining glucose. $MgCl_2$ was added as before. From the hydrolysed enzyme phosphoserine was identified in the same manner as previously described.

Our present method to show that the phosphorus atom is linked to the protein molecule consists in the isolation of a phosphorylated amino acid as phosphoserine from the hydrolysed protein. It therefore seems that the transfer of phosphorus in the hexokinase system is at least a two-step reaction. The phosphorus atom is first transferred from the donor molecule to the enzyme and secondly donated from the enzyme to the acceptor. We therefore consider that our results suggest that an enzyme-phosphate is an intermediate in the hexokinase reaction. Hexokinase and phosphoglucosmutase are examples of phosphoproteins functioning as transphosphorylases. It is highly possible that other phosphoprotein-enzymes may be engaged in the transfer of phosphorus in a similar way. This assumption is in line with the previously published high values for the incorporation of radioactive phosphate in the proteins from different cells².

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1. Ågren, G., de Verdier, C.-H. and Glomset, J. *Acta Chem. Scand.* **8** (1954) 503.
2. Ågren, G., de Verdier, C.-H. and Glomset, J. *Acta Chem. Scand.* **8** (1954) 1570.
3. Ågren, G., de Verdier, C.-H. and Glomset, J. *Acta Soc. Med. Upsaliensis* **9** (1955) 30.
4. Ågren, G., de Verdier, C.-H. and Glomset, J. *Acta Chem. Scand.* **9** (1955) 1041.
5. Boyer, P. D. and Harrison, W. H. *Mechanism of enzyme action*, Baltimore 1945, p. 658.
6. Najjar, V. A. and Pullman, M. E. *Science* **119** (1954) 631.
7. Beyer, R. E., Glomset, J. and Beyer, T. *Biochem. et Biophys. Acta* **18** (1955) 292.
8. Berger, L., Slein, M. W., Colowick, S. P. and Cori, C. F. *J. Gen. Physiol.* **29** (1945) 379.
9. Busch, H., Hurlbert, R. B. and Potter, V. R. *J. Biol. Chem.* **196** (1952) 717.
10. Siekevitz, P. and Potter, V. R. *J. Biol. Chem.* **215** (1955) 237.

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An Absolute Determination of the Rate of the Exchange Reaction between Cadmium Amalgam and Cadmium Cyanide Solutions

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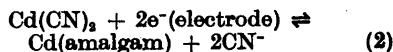
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The kinetics and mechanism of the heterogeneous exchange reaction at equilibrium electrode potential between cadmium amalgams and cadmium cyanide solutions containing sodium cyanide in excess was investigated by a radioactive tracer method in a previous work¹. Preliminary measurements showed that the rate of exchange in this system was not controlled by the diffusion of cadmium in the solution phase,² as was the case in perchlorate solutions³ or in solutions containing cadmium complexes of moderate strength³.

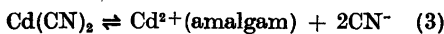
In order to attain sufficiently high accuracy in the isotopic exchange determinations, the main measurements were performed according to a procedure where the amalgam and the solution were shaken under reproducible conditions. For an activation-controlled exchange process a relationship of the following form should be obtained

$$r = k c^{1-a} q^a [CN^-]^{-4(1-a)} \quad (1)$$

where r denotes the rate of exchange, c the total concentration of cadmium in the solution, q the total concentration in the amalgam and k a constant. The exponent a is also a constant, fulfilling the condition $0 < a < 1$, and j is the number of cyanide ligands in the complex that predominates in the exchange process. Actually an expression of the form (1) for r was obtained in the investigation with the values $a = 0.34 \pm 0.02$ and $j = 2.2 \pm 0.1$. Thus the measurements indicated that under the existing concentration conditions the exchange reaction is activation-controlled and that the predominating reversible transfer reaction is the following one:



Since in the present case there is no transfer of electrons through the rigid part of the electrode double layer but a transfer of cadmium ions free from all ligands, the same process can also be denoted



By electrode impedance measurements, giving the exchange current density i_0 of the transfer step, Gerischer⁴ also has arrived at the result that $j \approx 2$ at low values of the cyanide ion excess, though the exponent 0.25 ± 0.02 of q diverges somewhat from our α -value.

In the investigation with the radioactive tracer it was possible to keep the average area of the interface constant, as was indicated by the measurements¹, but, since it was not possible by the method used to determine this area, the value of the constant k in eqn. (1) could not be obtained. However, a comparison between the resultant rate of the exchange r and the exchange current density i_0 , measured by Gerischer, is of great interest, and for this reason the present complementary investigation was performed.

The experimental procedure was the same as in the preliminary measurements of the previous investigation¹, *viz.* to let a stream of small amalgam droplets pass at a great velocity through the radioactive cadmium cyanide solution, containing sodium cyanide in excess. For a description of the apparatus used and other experimental details about the exchange measurements the reader is referred to an earlier work². In another paper³ it was shown how this apparatus was calibrated, so that the surface area of the droplets and the time of contact between the phases was known, and accordingly absolute values of the rate r were obtained.

Since the kinetic law (1) has already been verified, a determination of r for a single set of values of c , q , and $[\text{CN}^-]$ is sufficient to obtain the value of the constant k . In the experiments of the present investigation the concentrations $c = 7.0 \times 10^{-3}$ M, $q = 1.2$ M (1 % by weight) and $[\text{CN}^-] = 2.7 \times 10^{-2}$ M were used, and the ionic strength was 1 M with sodium perchlorate as an additional electrolyte. The rate of exchange measured was $r = 3.2 \times 10^{-8}$ mole cm^{-2} sec^{-1} . Thus we have

$$r = 1.5 \times 10^{-7} c^{0.86} q^{0.34} [\text{CN}^-]^{-0.46} \quad (4)$$

if r is expressed in mole cm^{-2} sec^{-1} and the concentrations in M. From the figures in

the paper by Gerischer⁴, it is found that for $c = 2 \times 10^{-3}$ M, $q = 2 \times 10^{-2}$ M, and $[\text{CN}^-] = 2 \times 10^{-2}$ M and at the ionic strength 5 M with sodium chloride as an additional electrolyte the exchange current density is $i_0 = 0.5 \times 10^{-3}$ A \cdot cm^{-2} . If i_0 is divided by $2F$, we get the rate 0.3×10^{-8} mole \cdot cm^{-2} \cdot sec^{-1} of the transfer reaction (3).

Now, if the expression (4) determined refers to the same partial reaction, we can use eqn. (4) for the calculation of the rate of this step for the last-mentioned values of c , q , and $[\text{CN}^-]$, even if the tracer method would possibly have given another kinetic law for the over-all exchange reaction for such a low value of the amalgam concentration q . Then we find $r = 0.4 \times 10^{-8}$ mole \cdot cm^{-2} \cdot sec^{-1} . Of course, this good agreement with the value obtained from i_0 is somewhat changed for varying values of c and q , since the exponents in eqn. (4) are not exactly the same as those found by Gerischer, but in any case the two methods give the same order of magnitude of r . Thus it is conclusively proved that for the ranges of concentrations used in the previous investigation¹ the over-all exchange reaction is controlled by the charge transfer between the phases.

In a paper about the kinetics of electrode processes, Vetter⁵ has discussed among other things the transfer reaction (3) above. He assumes that the two cyanide ions form a complex $(\text{CN})_2^-$ without any bond energy (in German "Begegnungskomplex" according to a notation of Weller⁶), which coordinates to the cadmium ion, leaving the amalgam. However, the assumption does not seem very plausible, especially if a corresponding conception is meant to be applicable to the transfer reaction for high values of the cyanide ion excess, where the third complex $\text{Cd}(\text{CN})_3^-$ predominates in this step (*cf.* Gerischer⁴).

According to the opinion of the present author it may be assumed that when the "naked" cadmium ion leaves the amalgam, two cyanide ions are coordinated consecutively without an intermediate coordination of water. First after this the more loosely bound water ligands are coordinated, forming an activated, hydrated complex with the central ion in the rigid part of the electrode double layer. For the reverse process, where all ligands are split off, we may assume the same stages, occurring in the reverse order.

Thus, since no other hydrated complex of the system is formed intermediately in the charge transfer reaction, it is justifiable to say that this step proceeds by way of the hydrated complex $\text{Cd}(\text{CN})_2$, though in all probability the ligands cannot be coordinated or split off simultaneously.

1. Fronæus, S. and Östman, C. O. *Acta Chem. Scand.* **8** (1954) 961.
2. Fronæus, S. *Acta Chem. Scand.* **7** (1953) 764.
3. Fronæus, S. *Acta Chem. Scand.* **8** (1954) 412.
4. Gerischer, H. Z. *Elektrochem.* **57** (1953) 604.
5. Vetter, K. J. Z. *Elektrochem.* **59** (1955) 596.
6. Weller, A. Z. *physik. Chem. N.F.* **3** (1955) 238.

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Chemically Induced Mutation and Sterility in Barley

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This paper gives some preliminary results from investigations concerning the induction, by chemical means, of mutations in higher plants. The experiments are complementary to treatments of the material with ionizing radiations¹, which seem rather unspecific since they always cause mutation as well as sterility, the latter effect especially at higher ion densities. A variation within wide limits of the linear energy transfer and irradiation conditions gives only small changes as regards relative frequencies of mutation types, possibly except the erectoides case². It was, therefore, our hope that an application of (a) chemically reactive compounds affecting biological materials differently, and (b) biochemically active compounds interfering with processes important to the synthesis of the gene material would, apart from an elucidation of the radiation induced mutation process, increase our possibilities to direct the mutation process.

In treatments of wet tissue with "radio-mimetic" chemical agents³, also when these are specifically nucleotoxic, it is extremely difficult to establish quantitative relationships based on a dosimetry. As compared to radiations, where active ions and radicals are formed anywhere in the tissue, diffusing molecules form gradients of change from outside towards inside of the tissue, cell, or cell nucleus. To some degree such difficulties might be overcome when dry resting seeds, which are in fact very insensitive to toxic agents, are treated with gaseous reactive chemicals. The imbibition of water and, in consequence, at least part of the final steps of the reactions will then follow afterwards. In the present study barley seeds containing 10–11 % H_2O were treated with a gas phase containing ethylene oxide, iodine, or chlorine. — Non-volatile compounds have to be applied in solution. Either resting seeds, or presoaked seeds, the germination process of which had been initiated by soaking in water for 24 hours, were immersed for different times in solutions of the agents studied.

All treatments were done at 20°C. Afterwards the seeds were sown in the field, and the mature plants obtained were analysed for survival, mutation, and sterility according to standard methods (cf. Ehrenberg and Nybom¹). Below, "sterility" is defined as the frequency of spikes with more than 10 % empty spikelets.

Tested compounds; results. The following subdivision as to the expected mode of action can be made (cf. Table 1):

(a) *Alkylating agents*, which after radicalization or ionization react with nucleophilic groups, such as amino and ionized acid groups. Effects of *mustards* have been described earlier^{1,4}. — *Ethylene oxide*, earlier found mutagenic in other organisms^{5,6}, was in part given, for 6 days, in the gas phase with 20 % air; in this treatment seeds survive if they contain less than 10 % H_2O ; partly presoaked seeds were treated for 2 hours with solutions (0.5% kills the seeds). — *Formaldehyde*, which, attacking amino and other groups, induces methylene groups and bridges, gives according to Favret⁷ mutations in barley. Under Favret's conditions, as well as in presence⁸ of H_2O_2 it was found inactive, however, although some sterility appeared.

(b) *Oxidizing agents.* *Chlorine* (24 h, 80 % being lethal) and *iodine* (saturated vapours, 48 hour's treatment lethal, 24 h used; washing in 10 % KI has to follow treatment) might oxidize (or substitute) almost everything.