A. nidulans heterotrophically in the dark by employing suitable carbon sources.

A. nidulans (14011) was initially grown in Allen's medium at 38 °C with continuous shaking. The culture was illuminated with day-light fluorescent lamps to yield 5000 lx intensity. Exponentially growing cells were harvested and inoculated in heterotrophic medium containing essentially all salts of Allen's medium with 0.5% sodium pyruvate and grown in the dark (figure 1). The cells grown heterotrophically in the dark were subcultured 2 or 3 times successfully and were found to be growing without production of pigments (figure 2). On transfer to autotrophic medium and light, these cells started synthesizing pigments again (figure 3).

The lack of pigment synthesis by *A. nidulans* in the dark is in contrast to the results reported with most blue-green algae grown in the dark. However *Plectonema boryanum* grown in the dark on glucose using a dialysis-flow through culture technique made no chlorophyll[15]. The results presented here clearly show that *A. nidulans* can be grown heterotrophically in the dark and is a good organism to study the regulation of pigment biosynthesis in procaryotes.

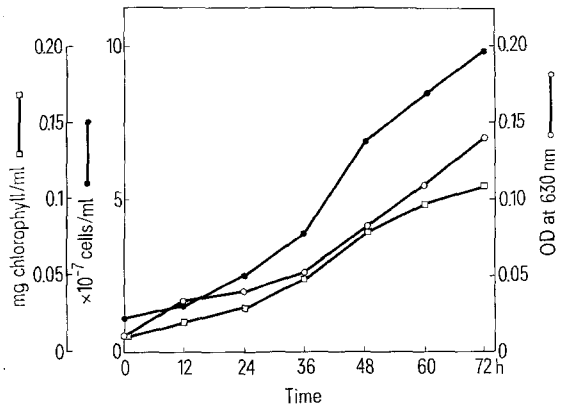

Fig. 3. Growth and pigment synthesis by dark grown cells in light. Cell multiplication (●—●); chlorophyll (□—□); and phycocyanin (○—○). Chlorophyll was extracted from the cells using 80% acetone and the pigment was estimated following the specific absorption coefficient of 82.0 at 663 nm. Phycocyanin was measured following the optical density of cells at 630 nm.

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Diethylpyrocarbonate interferes with lipid-protein interaction and glucose transport in the human red cell membrane[1]

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Summary. Diethylpyrocarbonate largely diminishes both discontinuities in red cell glucose transport and also in red cell membrane ANS fluorescence at about 17-20 °C.

Diethylpyrocarbonate has been successfully employed in protein chemistry for the characterization of reactive histidines which form N-ethoxyformyl-derivatives. Dependent on reactivity, the reagent may also modify lysine, tyrosine rests and sulfhydryl groups[2-5]. We report on experiments using the reagent in membrane biochemistry of the red cell, since in glucose transport imidazole rests of histidine, ε-amino groups of lysine have been found to play an essential role[6,7]. The investigations on glucose transport were supplemented by structural studies on red cell membrane and extracted membrane lipids, in order to achieve a better understanding of these residues in the architecture of the membrane.

Materials and methods. Erythrocytes of freshly collected human blood in ACD solution (11 g sodium citrate, 35 g glucose, 4 g citric acid with aqua bidest. ad 1000 ml) were preloaded with 200 mM glucose in isotonic NaCl solution by 4 washings. Incubation procedure and analytical methods have been described previously[8]. Red cell membranes were obtained by the method of Dodge et al.[9] as described previously[10,11]. The red cell membrane lipids were extracted according to the method of Dawson et al.[12]. Lipid estimations were carried out by the method of Zöllner and Kirsch[13], cholesterol was determined according to Watson[14].

Results. As seen in figure 1, glucose transport is influenced by temperature, and a discontinuity of log v_0 (v_0 = initial velocity without inhibitor) at around 20 °C is observed, as has been reported previously[15]. This discontinuity is correlated with the transition temperature, due to a structural change of the membrane lipids[10]. Since diethylpyrocarbonate is a protein reagent, an influence on the discontinuity of v_0 in glucose transport or on the transition temperature would not normally be anticipated. Nevertheless, there is a distinct change in slope of the straight line obtained for v_i (v_i = initial velocity in presence of inhibitor) of glucose transport for the whole temperature range. The discontinuity has disappeared. This interesting behaviour was fol-

lowed up by our experiments using red cell membrane and isolated red cell membrane lipids. In figure 2 we show the shift of pH of red cell membrane and extracted membrane lipids during reaction with diethylpyrocarbonate. The initial pH of 6.3 of red cell membrane is shifted within about 15 sec to pH 6.0 and within 2 min to about 5.5, ending up after 30 min incubation at about 5.0, indicating release of ethylcarbonate during the reaction. In comparison with red cell membrane lipids, the reaction does not proceed beyond about pH 6.0 after 30 min time. This indicates that diethylpyrocarbonate is bound to membrane lipid to much lower extent compared to red cell membrane. We were interested then to compare the influence of diethylpyrocarbonate on red cell membrane and extracted membrane lipid transition temperature. The results are shown in the figure 3, a and b. The known discontinuity at about 17-20 °C observed with ANS fluorescence[11] is severely restricted at 2.5 or 5 mM diethylpyrocarbonate in red cell membrane (figure 3, a). Also, the direction of the residual break is reversed compared to the control, as has been shown in ANS fluorescence experiments on red cell membrane using other types of inhibitors[11,16]. There is, however, still a similar break in both unmodified and modified red cell membrane lipids (figure 3, b).

Discussion. The results clearly show that reaction of diethylpyrocarbonate with the red cell membrane results in distinct changes in glucose transport over a temperature range, which is reflected in the diminution of the disconti-

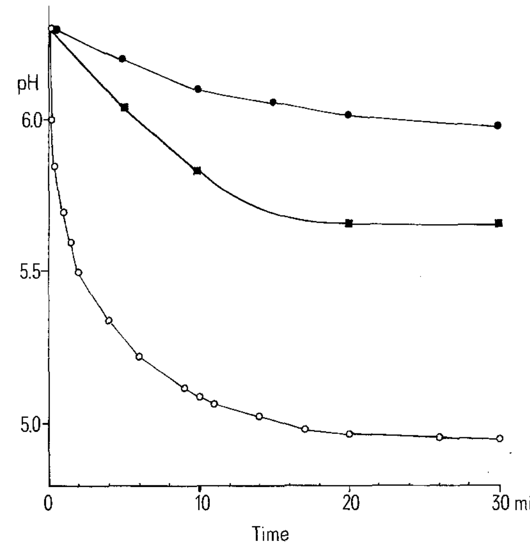

Fig. 2. Reaction of diethylpyrocarbonate with red cell membrane and with extracted red cell membrane lipids. 5 mg of membrane was suspended with 9 ml 0.9% NaCl and sonicated in an ice bath for 60 sec with a Branson S-75 sonifier at 4 A output. The initial pH of the suspension was 6.3. After addition of 2.5 mM diethylpyrocarbonate the pH changes were recorded. For the extracted membrane lipids in 0.9% NaCl the initial pH was 3.5 and was adjusted to 6.3 with a minute amount of base. ○, Red cell membrane, 2.5 mM diethylpyrocarbonate; ●, extracted membrane lipids, 2.5 mM diethylpyrocarbonate, 1.56 mg total lipid, 30% cholesterol in 5 ml 0.9% NaCl; ■, extracted membrane lipids, 5.0 mM diethylpyrocarbonate, 1.82 mg total lipid, 30% cholesterol in 5 ml 0.9% NaCl.

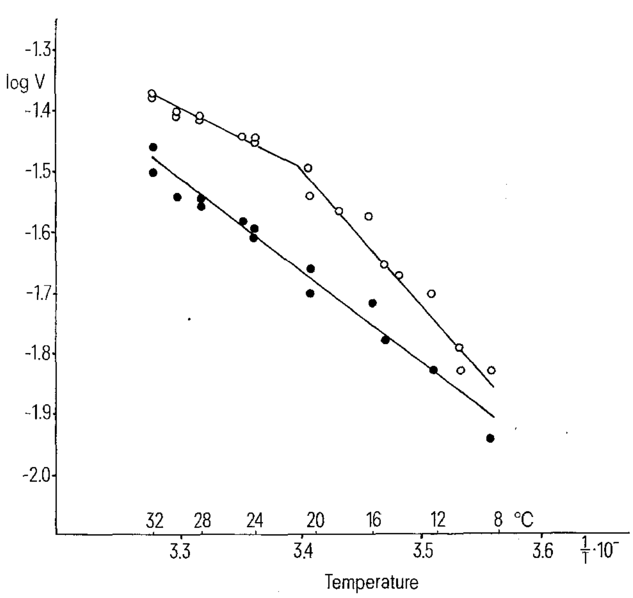

Fig. 1. Arrhenius plot of the effect of diethylpyrocarbonate on the velocity of glucose uptake. Experiments were carried out with 4 different blood donors. Red cells, preloaded with 200 mM glucose were incubated with 0.9% NaCl and 0.04 mM ^{14}C glucose at the temperatures indicated. The logarithm of the velocities (μmole/ml ery · sec) was plotted against the reciprocal value of °K. The regression lines are: control 8-20 °C, y = −2.26x + 6.2; correlation coefficient r = 0.97; 20-32 °C, y = −1.02x + 1.99; r = 0.96; 2.5 mM diethylpyrocarbonate: 8-32 °C y = −1.54x + 3.57; r = 0.95, ○, Controls; ●, 2.5 mM diethylpyrocarbonate; experimental points are means of 3 determinations.

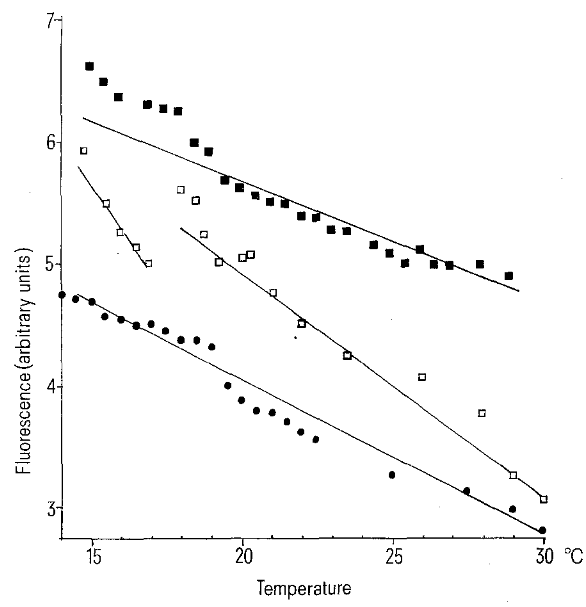

Fig. 3. *a* 1-anilino-naphthalene-8-sulfonate (ANS) fluorescence of red cell membrane over a temperature range. 10 mg of membrane was sonicated with 9 ml 0.9% NaCl (see figure 2). The suspension was diluted so that a final protein concentration of 0.3 mg/ml was obtained. Thereafter, sodium phosphate buffer pH 7.0 was added to a final concentration of 0.01 M. The ANS concentration was 40 μM. Fluorescence was measured with a Farrand Mark I spectrofluorometer, excitation was at 380 nm, maximal emission was found at 462 nm. The maxima were recorded 5 times, so that the points derived from these measurements represent means of apparative deviation. Incubation with diethylpyrocarbonate was 30 min at 23 °C, thereafter, phosphate buffer pH 7.0 was added. □, Control; ●, 2.5 mM diethylpyrocarbonate; ■, 5.0 mM diethylpyrocarbonate. The lines were calculated by the method of least squares.

nuity of red cell membrane ANS fluorescence. In contrast, the extracted membrane lipids are not influenced in the same way by the reagent. Since the discontinuity in this temperature region is due to the membrane lipids[10,17-20], it must be concluded that the reagent interfered with lipid-protein interaction. Diethylpyrocarbonate will react with histidine, tyrosine and lysine in proteins[2]. SH groups are also possible candidates for the reaction, depending on their reactivity. We did not find, however, any alteration in inhibition of glucose transport by diethylpyrocarbonate when 5 mM cysteine was present during incubation (not shown). Also, membrane SH groups were considered to be probably not essential for glucose transport activity[7]. Since there was also no difference detected between the controls and modified membrane when spectrophotometric measurements were carried out above 270 nm, extensive reaction of tyrosine rests may be excluded (not shown). The remaining rests are histidine-imidazole and lysine. On the one hand, both have already been implied to be probably essential for glucose transport[6,7]. On the other hand, basic amino acids have been considered for a while to be possible candidates in polar lipid-protein interaction in plasma membranes[21]. The polar head of lipid phosphate may interact electrostatically with the basic rest of the amino acid. It is known, furthermore, that lipid transition is dependent on liberty of motion of the polar head groups and also on an increase in mobility of the fatty alkyl chains. Thus, a change in mobility of the head groups induced by the reagent's interaction with basic amino acid residues may decrease activation energy below and increase above transition temperature (figure 1). Below the transition temperature, there is an increase of disorder of the system in the presence of diethylpyrocarbonate. Above the transition temperature, where we already observe an increase of disorder, the effect of the reagent is opposite.

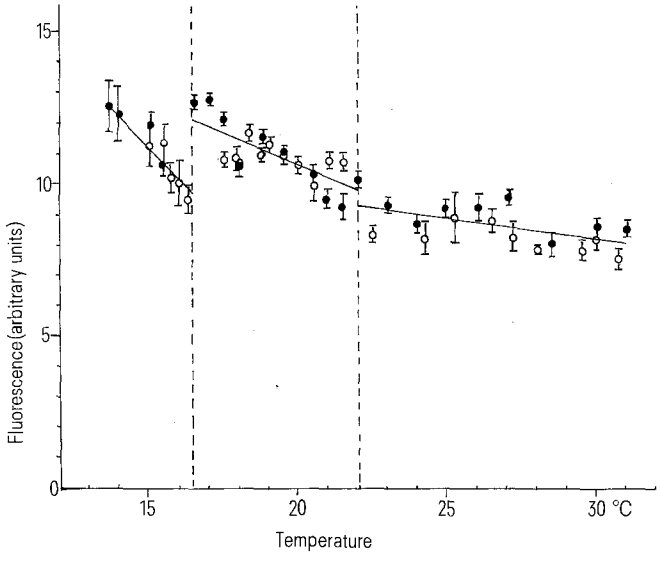

Fig. 3. *b* ANS fluorescence of extracted red cell membrane lipids over a temperature range. 0.78 mg of total lipid (30% cholesterol) was suspended with 2.4 ml 0.9% NaCl and sodium phosphate buffer, pH 7.0 to a final concentration of 0.01 M was added. The ANS concentration was 5.6 µM. Incubation with diethylpyrocarbonate similar to figure 3, a. The points±SD represent means of 10 recordings (see figure 3, a). ○, Control; ●, 5 mM diethylpyrocarbonate. The lines were calculated by the method of least squares.

1 We are very much indebted to Dr K.-H. Röhm for valuable discussions of this paper. This work was supported by the Deutsche Forschungsgemeinschaft.
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Adhesion of human red blood cells to polystyrene. Influence of sodium chloride concentration and of neuraminidase treatment

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Summary. The adhesion on polystyrene of glutaraldehyde-fixed human red blood cells was found to increase with NaCl concentration. Half of the maximum of irreversible adhesion was obtained in 2.2 mM NaCl for neuraminidase-treated cells and in 5.5 mM NaCl for the untreated cells.

As Curtis[1] pointed out, the classical DLVO theory of double layer interaction developed by Derjaguin and Landau[2] and by Verwey and Overbeek[3] predicts that cell adhesion can occur in 2 minima of the potential energy of interaction. At some finite distance, where the surface does not come into molecular contact, an equilibrium is reached between electro-dynamic attractive and electrostatic repulsive forces (secondary minimum). At smaller distance there is a net energy barrier. Once overcome, the theory predicts another minimum (primary minimum). Both the height of