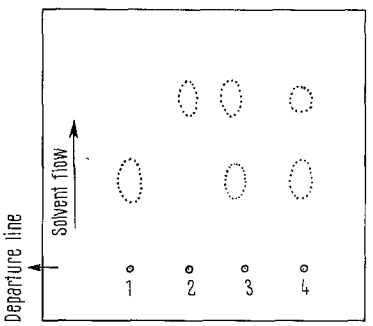
Phosphoglucomutase was prepared from potatoes by the method of RUSSEL[7]. The reaction mixture consisted of 1. 0.4 ml of phosphoglucomutase preparation, 2. 0.2 ml of 0.1 *M* cysteine solution (pH 7.5), 3. 0.2 ml of 0.1 *M* citrate buffer (pH 7.4), 4. 0.3 ml of either double distilled water or inhibitor solution, 5. 0.4 ml of 0.06 *M* glucose-1-phosphate solution. The reaction mixtures were incubated at 37 °C for different periods and the reaction stopped by the addition of 1 ml of 5 *N* sulfuric acid and volume made to 5 ml with distilled water. The reaction tubes were placed in a boiling water-bath for 3 min and the liberated phosphorus was determined by the method of FISKE and SUBBAROW[6]. The incubation mixtures were also subjected to TLC for resolution of sugar phosphates by the method of DAVIDSON and DREW[8].

Effect of inhibitor on phosphoglucomutase activity

Incubation period (min)	Inorganic phosphorous of glucose-6-phosphate formed from glucose-1-phosphate in the reaction mixture (mg)	
	With inhibitor	Without inhibitor
4	0.21	0.20
6	0.20	0.25
8	0.24	0.29
12	0.25	0.35
15	0.25	0.47



Representation of thin layer chromatogram developed by the method of DAVIDSON and DREW[8]. Initial spots at the departure line were 1. glucose-1-phosphate solution; 2. glucose-6-phosphate solution; 3. reaction mixture without inhibitor solution, incubated for 15 min; 4. reaction mixture with inhibitor solution, incubated for 15 min. Faint dotted circles indicate weak spots.

Results. The inhibitor is dialyzable and soluble in water, benzene, chloroform and di-ethyl ether. It can be concentrated by extraction in these solvents, followed by in-vacuo evaporation at low temperature. It did not show any effect on starch phosphorylase activity when incubated up to 10 min. Data reported in the Table and thin layer chromatography analysis (Figure) showed partial inhibition of conversion of glucose-1-phosphate to glucose-6-phosphate when reaction mixtures contained inhibitor solution.

Discussion. Simultaneous occurrence of sucrose[2] and fructosan in onion bulbs presents a dilemma, since known systems for the synthesis of sucrose require glucose-1-phosphate[9] and sucrose is a substrate for fructosan biosynthesis[10,11]. In view of the presence of phosphoglucomutase inhibitor, the alternate pathways may exist in this plant for the synthesis of sucrose from fructose phosphates and UDPG[12–14]. Then the biosynthesis of starch from sucrose may be inhibited owing to the presence of phosphoglucomutase inhibitor, as it is one of the enzymes involved in its synthesis and sucrose is utilized for fructosan biosynthesis. The speculation is also tenable because synthesis of fructosans does not require glucose-1-phosphate.

Zusammenfassung. Aus Zwiebelknollen (*Allium cepa* Linn.) wurde ein Hemmstoff der Phosphoglukomutase isoliert.

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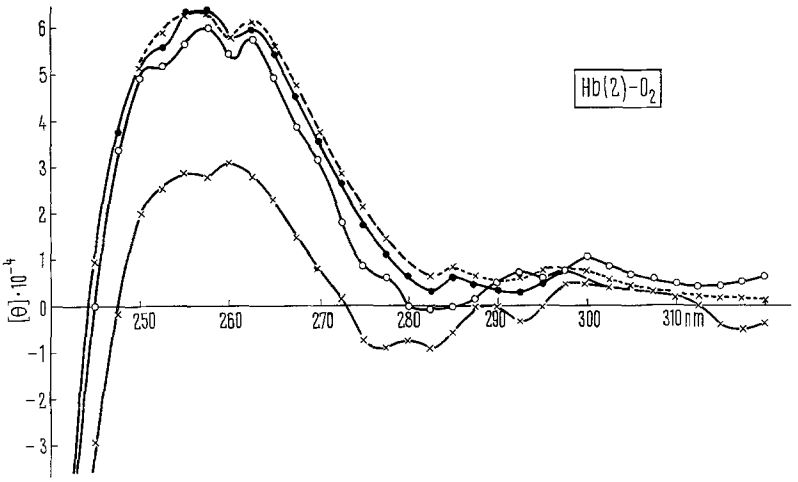
[7] P. RUSSEL, Fd. Res. Inst. Stud. Stanford Univ. *32*, 381 (1967).
[8] I. W. F. DAVIDSON and W. G. DREW, J. Chromatogr. *21*, 319 (1966).
[9] J. F. TURNER, Nature, Lond. *172*, 1149 (1953).
[10] I. S. BHATIA, M. N. SATYANARAYANA and M. SRINIVASAN, Biochem. J. *61*, 171 (1955).
[11] I. S. BHATIA, T. SATYANARAYANA and K. V. GIRI, Ind. Sci. Congr. Abstr., Part IV, 135 (1959).
[12] C. E. CARDINI, L. F. LELOIR and J. CHIRIBOGA, J. biol. Chem. *214*, 149 (1955).
[13] T. YAMAHA and C. E. CARDINI, Arch. Biochem. Biophys. *86*, 127 (1960a).
[14] T. YAMAHA and C. E. CARDINI, Arch. Biochem. Biophys. *86*, 133 (1960b).

Studies on the Behaviour of Circular Dichroism of Different Haemoglobins in the 260 nm Region

In 1968 we found that complexes of human methaemoglobin and metmyoglobin differ in optical activity in the soret and 260 nm region[1]. The values of ellipticity of the soret region compared with those of the 260 nm region show an opposite tendency: the ellipticity at 260 nm increases with the low-spin character of the complexes while in the soret region the values decrease with increasing low-spin character. We conclude from this finding that there must be different chromophores which produce cotton effects at 260 nm and in the soret region[2]. Moreover, in both regions, significant species-dependent differences are to be observed. From correlations between the affinity of different ligands of haemoglobin (alcylisocyanides) and of methaemoglobin with

the ellipticity in the 260 nm region, it is considered that the intensity of the ellipticity at 260 nm reflects interactions between the prosthetic group and the protein, especially with hydrophobic parts of the molecule[2,3].

Now CD measurements were made on different haemoglobins in the 260 nm region with greater resolution of the dichrographe (Jouan, type 185). We found that the broad CD-absorption band in the 260 nm region can be resolved in about 3–4 distinct maxima. In the Figure CD-absorption spectra of oxyhaemoglobin of 4 different species are to be seen. The resolution of the broad CD-band differs in dependence of the species investigated. Especially in the case of foetal and adult human haemoglobin well resolved CD-spectra with 4 maxima can be distinguished (Figure).



CD-spectra of ●—● adult human-, ×---× bovine-, ○—○ fetal human-, ×—× carp oxyhaemoglobin. Ordinate: Ellipticity on molar haem basis; 23 ± 1.5 °C; 1/15 *M* phosphate buffer, pH 7.0; concentration 4×10^{-4} val/l; cuvette 5×10^{-3} cm.

In 1965[4] we analyzed the position of the absorption bands of phenylalanine in Hb-A. In this case we took methaemoglobin because of better resolution of the spectra. Comparing now the position of the maxima in CD-curves with the absorption maxima in optical spectra in human haemoglobin shows them to be in good agreement.

Further we observed that bovine apohaemoglobin exhibits a positive cotton effect in the 260 nm region.

From these results we conclude that for the origin of the CD-bands in the 260 nm region, transitions of phenylalanine residues are also responsible. According to the theory of Tinoco[5] two reasons may be due to the cotton effects in 260 nm region; the coupling of transition of the haem chromophore which has a small absorption band in this region with the aromatic amino acid residues. These interactions are also responsible for the origin of the optical activity in the soret region[6]. The second contribution to the CD-band in 260 nm region comes from coupling of aromatic side chain transitions and the interaction of these with the haem group. The major factor of the observed rotational strengths in the 260 nm region is the interaction of the haem group with aromatic side chain transitions because of the strong dependence of this CD-absorption on the spin of the haem iron and the diminished CD-band after removing the haem group. The fine structure of the CD-band in 260 nm region can only be produced by the second point because the fine structure reflects the vibrionic structure of the UV transitions of the perturbed aromatic chromophores.

From absorption measurements of phenylalanine in haemoglobin, it is known[4] that the resolution is lowered by overlapping absorption of other aromatic amino acids. Therefore the CD-band can be resolved only with difficulty in single peaks and distinguished only at several species.

Zusammenfassung. CD-Messungen mit hoher Auflösung an Hämoglobinen verschiedener Spezies ergaben eine Aufspaltung der breiten Absorptionsbande bei 260 nm in 4 einzelne Maxima. Diese stimmen mit der Bandenlage des Phenylalanins im Absorptionsspektrum vom Hämoglobin gut überein und werden daher diesem Chromophor zugeordnet.

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Absorption spectra Hb-A Position of Phe-bands Hb(3)	CD spectra Hb(2) Hb-A CD-maxima Hb(2)-CO	Hb(2)-O_2
254.0 nm	254.0 nm	251.0 nm
259.7 nm	258.5 nm	257.5 nm
265.7 nm	263.0 nm	262.5 nm
269.5 nm	270.0 nm	270.0 nm

1 K. Ruckpaul and H. Rein, Dte GesundhWesen *24*, 1197 (1969).
2 K. Ruckpaul, H. Rein and F. Jung, Acta biol. med. german. *24*, 445 (1970).
3 K. Ruckpaul, H. Rein and F. Jung, Acta biol. med. german. *24*, 33 (1969).
4 F. Jung, G. Stopp and K. Ruckpaul, Nature *207*, 990 (1965).
5 I. Tinoco jr., Adv. Chem. Phys. *4*, 113 (1962).
6 M.-C. Hsu and R. W. Woody, J. Am. chem. Soc. *91*, 3679 (1969).