off during a dive resulted in a return of bradycardia (Fig. 1B). When the pacer was turned on during diving, the ventricular response could be captured at about 85 impulses per minute (Fig. 1C) and then regulated at any desired rate above that required for ventricular capture. Such controlled pacing during diving was sustained without distress to the animals for periods in excess of 6 minutes, a period of diving not compatible with survival if arterial constriction is prevented (*6*).

Heart rates comparable to the nondiving rate (120 to 150 beats per minute) were compatible with prolonged diving but generally resulted in a gradual decrease in systolic and diastolic aortic pressure and in pulse pressure. Rates greater than 150 beats per minute produced a sharp progressive decrease in aortic pressure (Fig. 1, C and D). If the rate was then decreased, aortic pressure and pulse pressure increased progressively (Fig. 1D). At rates of about 100 to 120 beats per minute, aortic pressure remained fairly constant (Fig. 1C). During diving the aortic blood pressure could be manipulated simply by altering the heart rate by means of the intracardiac pacer. Occasionally, during diving with pacing, ventricular escape from the pacer occurred. The next ventricular response was accompanied by an increase in aortic pressure (Fig. 1C).

Arteriograms performed during diving with pacing demonstrated the same degree of profound arterial constriction that occurred during a normal dive (*4*). The role of the arterial constrictor response in oxygen conservation seems clear. Profound constriction of arteries perfusing all peripheral tissues that were studied (except brain and possibly heart) (*4*) prevents further delivery of oxygen to these sites, thus conserving available stores of oxygen for the oxygen-dependent central nervous system. Peripheral tissues affected by the arterial constrictor response convert to the process of anaerobic glycolysis when the oxygen already present in these tissues is consumed (*7*).

Heretofore the precise role of bradycardia during diving has not been clearly demonstrated. Failure of the normal dive response in the harbor seal generally produces death from anoxia within 4 minutes of diving. The present finding that the seal can remain submerged, with an intact arterial constrictor response but without bradycardia, for more than 4 minutes demonstrates that the arterial constrictor response is the more important circulatory adaptation during diving. The finding that the arterial constrictor response occurs without initial bradycardia suggests that arterial constriction is not mediated in response to bradycardia. It appears that bradycardia is not essential for the conservation of oxygen or for the institution of the dive response.

Previous studies in the seal indicate a reduction in heart rate with a relatively proportional decrease in cardiac output during diving as the perfused vascular bed is decreased. Although there are no precise data concerning the acute changes in venous return upon diving, a number of factors might be anticipated to reduce venous return. These include (i) the reduced cardiac output per se, (ii) pooling of blood in the unusually large hepatic sinuses and numerous abdominal veins of this animal, (iii) reduction in pressure gradient between extrathoracic and intrathoracic compartments produced by immersion of the whole animal in water, (iv) loss of respiratory pumping of blood into the thorax during the apnea of diving, and (v) possible changes in venomotor tone in the extrathoracic venous system. Thus, when the heart rate is increased above a critical level there is a decrease in aortic pressure, presumably reflecting impairment of diastolic filling caused by the decrease in venous return. The sudden increase in aortic pressure, when there is ventricular escape from the pacer during diving, is consistent with this hypothesis.

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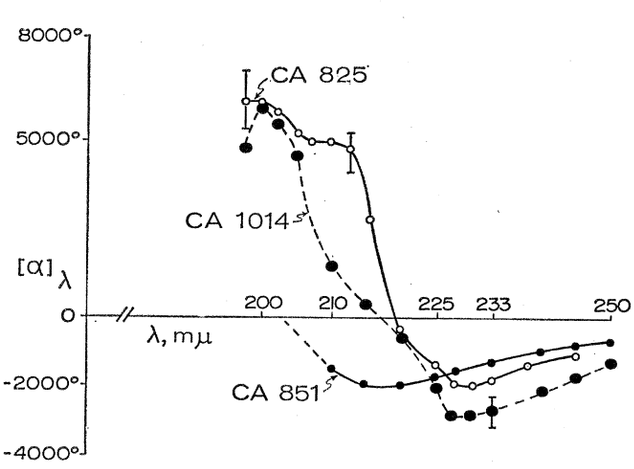
Conformation of Blood-Group and Virus Receptor Glycoproteins from Red Cells and Secretions

Abstract. *Optical rotatory dispersion of human blood-group and virus receptor glycoproteins from erythrocytes and secretions was studied in the far-ultraviolet. Erythrocyte membrane blood-group glycoproteins, with potent virus receptor activities, contained significant alpha-helical and extended beta conformations, whereas the glycoproteins of secretions were largely disordered. These conclusions were supported by determination of the Moffitt constants* (b_0) *and by measurements of circular dichroism.*

Blood-group substances seem to have been studied by optical rotatory dispersion by only Beychok and Kabat (*1*). They limited investigation to the main representatives of the first human blood-group system, the A,B, and H(O) antigens isolated from ovarian cyst fluid. These glycoproteins contain 75 to 80 percent carbohydrate, including approximately 30 percent hexosamine, and 20 to 25 percent peptide; their molecular weights are around 300,000 (*2*). The substances showed weak optical rotatory dispersion, and the curves had a low negative extremum near 220 mμ. Beychok and Kabat attribute the negative extremum at least in part to the *N*-acetyl(2-acetamido) group of hexosamines, especially β-linked *N*-acetyl-D-glucosamine; they found no evidence of α-helical conformation in these ABH(O) substances from epithelial secretions (*1*).

The main antigens of the second human blood-group system to be discovered (*3*), the M and N substances, have been isolated from human erythrocyte membranes and characterized as glycoproteins (see *4*). They are among the most powerful inhibitors of influenza virus hemagglutination yet described (*5*). These glycoproteins contain between 50 and 55 percent carbohydrate, including 12 to 25 percent *N*-acetylneuraminic acid and 10 to 13 percent *N*-acetylated hexosamines; they have been obtained in homogeneous form in various stages of aggregation from molecular sizes of 12×10^6 down to approximately 3×10^4. Blood-group and antiviral activities increase in parallel with increase in molecular size. Harsh treatment readily disaggregates the larger molecules into smaller ones consisting of units that are multiples of 30,000 (*5*).



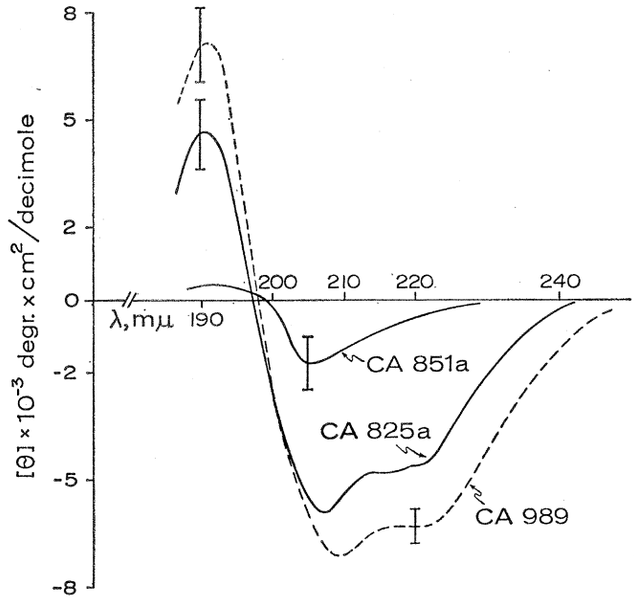


Fig. 1 (left). Far-ultraviolet optical rotatory dispersion spectra of human blood-group antigens and virus receptors. Ca 825, NN blood-group antigen and virus receptor from erythrocyte membranes. Ca 1014, MM blood-group antigen and virus receptor from erythrocyte membranes. Ca 851, Vg blood-group antigen and virus receptor from meconium. The glycoproteins were dissolved in 0.01*M* sodium phosphate buffer, *p*H 7.2. The concentration of the substances was from 0.020 to 0.038 percent; the optical path was 0.50, 0.20, or 0.10 cm. A symmetric angle at 5° was used for the oscillating prism. Fig. 2 (right). The ellipticity curves of the erythrocyte NN blood-group antigen Ca 825a, the erythrocyte MM blood-group antigen Ca 989, and the meconium antigen Ca 851a. The solvent and optical paths were the same as in Fig. 1; the concentrations were from 0.008 to 0.032 percent. The curves were calculated from at least three dichroism recordings for each sample. All runs were made with the most sensitive scale setting of 10^{-4} differential dichroic absorbance per 1 cm on the recording. The mean residual weights used in calculating the molar ellipticities were 177 for Ca 825a and Ca 989 and 226 for Ca 851a.

In order to elucidate the conformation of these cell membrane components, their dispersion in the ultraviolet spectral zone of 190 to 300 mμ was studied and compared with those of two blood-group antigens isolated from human secretions (*5*, *6*) and a blood-group H(O) specific polysaccharide from the tree *Sassafras albidum* (*7*). The improved Rudolph model RSP-3-4 recording spectropolarimeter was used (*8*). All samples were dissolved and measured as indicated in Figs. 1 and 2. The Moffitt constants, b_0, were determined from measurements at 240 to 350 mμ, with the use of λ_0 of 216. The refractive index and residual weight corrections were disregarded, since they do not significantly affect the results.

The most striking results of the optical rotatory dispersion measurements are shown in Fig. 1. Samples Ca 1014 and Ca 825 have some conformational order. The human erythrocyte blood-group MM specific antigen Ca 1014 is a very potent virus receptor, molecular weight 1.8×10^6 (*5*); some α-helix content is indicated by its peak at 200 mμ and the trough near 230 mμ. However, since the trough extends to about 227 mμ, some β structure also may be present. Ca 825, a human blood-group NN specific and virus receptor glycoprotein, molecular weight 5.95×10^5, isolated from erythrocyte membranes (*5*), has similar conformational order. The inflection point of both curves occurs at 216 to 218 mμ. The inferences drawn from the shape of the curves are supported by the measured b_0 values: −88 and −50 for Ca 1014 and Ca 825, respectively.

Work on other blood-group MM specific substances isolated from erythrocytes showed that disaggregation products of the very high-molecular-weight glycoprotein gave rotatory dispersion curves indicating lesser conformational order. Thus the b_0 of the disaggregation product of Ca 1014, specimen Ca 990-Ki (molecular weight 3.6×10^4), was zero, the trough at 230 mμ was shallower, and the peak at 200 mμ was lower.

The conclusions about the conformation of the MM and NN substances, which were based on the rotatory dispersion data, were supported by measurements of circular dichroism with the highly sensitive Jasco-Durrum CD-SP dichrograph [for general information on circular dichroisms see, for example, Beychok (*9*)]. Some results are shown in Fig. 2 in the form of ellipticity curves for the MM glycoprotein Ca 989 (molecular weight 1.8×10^6, closely similar to Ca 1014 but somewhat polydisperse) and the NN glycoprotein Ca 825a (symbol "a" designates that the substances differ from those without it by having been freeze-dried and stored, with buffer salts, for considerable periods of time). The presence of dichroic extrema at 214 to 222 mμ, 207 to 210 mμ, and 190 to 194 mμ is in agreement with the composite conformational organization of the erythrocyte membrane antigens.

An accurate calculation of the α-helix content is impossible, because of the overlap of the various effects produced by the backbone conformations and *R*-groupings of amino acids and carbohydrates. Also, we found that *N*-acetylneuraminic acid, which is a major component of the MM and NN substances, shows a weak positive Cotton effect at about 195 mμ and Beychok and Kabat noted that *N*-acetylated amino sugars, also present in these substances, have weak negative effects in the far-ultraviolet (*1*). Estimates of the α-helix content yield 8 to 16 percent of this conformation in the erythrocyte antigens.

Dispersion studies of the two glycoproteins isolated from human secretions showed them to be largely disordered. Ca 851, molecular weight 5.2×10^6, a glycoprotein from meconium has virus and blood-group receptor activities. It contains approximately 85 percent car-

bohydrate, including about 31 percent *N*-acetylhexosamines and 10 percent *N*-acetylneuraminic acid; the remainder is peptide (*5*). Figure 1 shows that the far-ultraviolet curve of Ca 851 has a minimum around 216 mμ, thus it probably is largely disordered. It is devoid of α-helix but might have a low content of the β conformation (*10*). The nonhelical structure of this glycoprotein is confirmed by circular dichroism measurements (Fig. 2) and by its b_0 value, which was zero. A blood-group H(O) specific ovarian cyst glycoprotein (Ca 1047, $S_{20,w} = 7.6S$ at a concentration of 0.63 percent) which contains about 35 percent *N*-acetylhexosamine yielded a dispersion curve similar to that of Ca 851; its b_0 value was + 63, and thus it is also a largely disordered chain. Featureless rotatory dispersion and circular dichroism curves running near base lines were observed also with a blood-group substances and virus recep- from *Sassafras albidum* which is free of peptide and hexosamine and has a molecular weight of 250,000.

The findings on the two substances from human secretions are in general agreement with those by Beychok and Kabat (*1*). Our observations on the blood-group substances and virus receptors from cell membranes indicate, however, that they possess ordered structures to a significant degree, thus they differ from the glycoproteins in secretions. These findings are also important in considerations of cell membrane structure. Ours appears to be the first observation of the α and β conformations in blood-group glycoproteins, and the conformation can be attributed to the peptide parts of the molecules. Also, these studies tempt one to conclude that biological activities of the important cell-membrane glycoproteins depend not only on their terminal carbohydrates but in part on the conformation of their peptide constituents, since the disaggregation product Ca 990-Ki shows not only a lesser conformational order but also considerably lower blood-group and antiviral activities than do Ca 1014 and Ca 989, the much larger aggregates from which it is derived (*5, 11*).

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Hydroxyindole-O-Methyl-Transferase Activity: Effect of Sympathetic Nerve Stimulation

Abstract. *Stimulation of the preganglionic nerve trunk to the superior cervical ganglion causes a reduction in pineal hydroxyindole-O-methyl-transferase levels which is time-dependent. The results provide direct evidence for a role of afferent input in the control of pineal enzymatic activity.*

There is considerable evidence to support the concept that the level of pineal hydroxyindole-*O*-methyl-transferase (HIOMT), as well as the levels of other pineal constituents, is under neural control (*1*). A circadian rhythm in rat pineal HIOMT has been described in which enzyme activity falls during hours of light and rises during hours of darkness. Blinding by bilateral orbital enucleation or placing the animals in constant light or darkness interrupts rhythms of all pineal constituents studied to date with the exception of serotonin, the rhythm of which apparently persists in blinded animals or animals kept in darkness. Moreover, in animals exposed to continuous light for 7 to 30 days, the levels of HIOMT in the pineal are low, while in those rats which have been kept in darkness or blinded there is an elevated level of the enzyme. This light-dark difference can be abolished by lesions producing bilateral degeneration of the inferior accessory optic tract (*2*) or by superior cervical ganglionectomy. The postganglionic sympathetic fibers arising from the superior cervical ganglion are thought to supply the pineal gland in the rat its sole source of innervation (*3*). Therefore, neural control of HIOMT is undoubtedly mediated via these fibers. In order to obtain direct evidence for such control of pineal enzyme activity, we have studied the effect of stimulating the preganglionic cervical sympathetic fibers on the HIOMT activity in the rat pineal gland.

We assayed HIOMT activity at optimum substrate concentrations, using a modification of the procedure of Axelrod *et al.* (*1*). Individual pineals were homogenized in 0.5 ml of 0.05*M* phosphate buffer (*p*H 7.9). The entire homogenate or an aliquot of it was transferred to a 15-ml glass-stoppered centrifuge tube containing 687 mμmole of *N*-acetyl-serotonin, 44 mμmole of purified *S*-adenosyl-methionine (*4*), and 4 mμmole of methyl-C^{14}-*S*-adenosyl-methionine (50 μc/μmole, New England Nuclear). The final volume was 850 μl. After 30 minutes' incubation, at 37°C, 1 ml of 0.2*M* borate buffer, *p*H 10, and 10 ml of chloroform were added. The tube was shaken for 5 minutes, the aqueous phase removed, and the chloroform washed with 1 ml of buffer. Five milliliters of the chloroform extract was transferred to a counting vial, the chloroform evaporated, the residue taken up in 1 ml of ethanol, and the radioactivity measured after the addition of 20 ml of phosphor. A control incubation in which 0.5 ml of *p*H 7.9 buffer was substituted for the enzyme was run concurrently. These blanks were found to be equivalent to those which contained boiled pineal homogenate. The data are expressed as micromicromoles of melatonin formed per pineal per hour.

Rats (90-day-old males of the Holtzman strain) were anesthetized with ether, and bilateral orbital enucleations