Jardetzky, C. D., and Jardetzky, O. (1960), *J. Am. Chem. Soc. 82*, 222.
Li, N. C., Scruggs, R. L., and Becker, E. D. (1962), *J. Am. Chem. Soc. 84*, 4650.
Moll, H., Schneider, P. W., and Brintzinger, H. (1964), *Helv. Chim. Acta 47*, 1837.
Schneider, P. W., and Brintzinger, H. (1964), *Helv. Chim. Acta 47*, 1717.
Zubay, G., and Doty, P. (1958), *Biochim. Biophys. Acta 29*, 47.

Sequential Periodate Oxidation of the α_1-Acid Glycoprotein of Human Plasma*

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ABSTRACT: The sequential periodate oxidation of sialic acid free α_1-acid glycoprotein of human plasma and of a derivative obtained by treatment with *Diplococcus pneumoniae* neuraminidase and β-galactosidase is described. After three degradations of both products only 2-acetamido-2-deoxyglucose units remain attached to the polypeptide chain, implicating these monosaccharide units in the linkage between carbohydrate and protein moieties. From the content of 2-acetamido-2-deoxyglucose, a minimal value of 5–6 carbohydrate–protein linkages was estimated. The sequential periodate oxidation indicated also for each molecule of glycoprotein the presence of 8–9 chains containing *O*-β-D-galactopyranosyl-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2- or -6)-D-mannopyranosyl residues, five chains containing *O*-β-D-galactopyranosyl-(1→6)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2- or -6)-D-mannosyl residues, with 30 to 40% of the chains possessing branch points at C-3 or C-4 of the mannosyl residues, as well as the presence of 2-acetamido-2-deoxyglucosyl-2-acetamido-2-deoxyglucosyl residues.

The α_1-acid glycoprotein (orosomucoid) of human plasma (Weimer *et al.*, 1950; Schmid, 1950, 1953) has a content of approximately 45% of carbohydrate which is linked to a polypeptide moiety. Previous studies undertaken in this laboratory have been concerned with the number and the structure of the carbohydrate chains and with the mode of linkage between the carbohydrate and protein moieties of the macromolecule (Eylar and Jeanloz, 1962a,b; Jeanloz and Closse, 1963; Hughes and Jeanloz, 1964a,b). Present evidence suggests that the α_1-acid glycoprotein contains a carbohydrate moiety consisting of a total of 16 chains. Each chain possesses a *N*-acetylneuraminic acid residue as nonreducing and terminal group with the exception of two chains, where L-fucose replaces *N*-acetylneuraminic acid. Some of the chains are terminated by the sequence *O*-(*N*-acetylneuraminyl)-(2→4)-*O*-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucose. The inner core of the polysaccharide moiety is of unknown structure and contains several D-mannose and 2-acetamido-2-deoxy-D-glucose units.

In order to obtain information on the chemical structure of the core, and on the linkage between the carbohydrate and protein moieties, the α_1-acid glycoprotein and two derivatives have been subjected to sequential periodate oxidation, a method based on the observations of Smith and co-workers (Smith and Unrau, 1959; Goldstein *et al.*, 1959; see also Whelan, 1960). Periodate oxidation of a polysaccharide results in the cleavage of available glycol groups and the production of a polyaldehydic structure. Subsequent reduction of the aldehyde groups with sodium borohydride produces a polyalcohol containing acetal linkages, which are easily hydrolyzed under conditions which do not cause accompanying cleavage of the remaining glycosidic bonds. Cleavage of the acetal linkages exposes new monosaccharide units which may be susceptible to further oxidation. Analysis of the monosaccharide units destroyed after each application of the reaction sequence provides a basis for deducing a partial structure for the intact polysaccharide.

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Materials and Methods

α_1-Acid Glycoprotein and Derivatives. The α_1-acid glycoprotein was prepared as described by Schmid (1953) from pooled human plasma and showed the properties previously described (Hughes and Jeanloz, 1964a). The *N*-acetylneuraminic acid units were removed from the α_1-acid glycoprotein by hydrolysis with 0.04 N sulfuric acid for 1 hr (Eylar and Jeanloz, 1962b). The treatment removed completely the sialic acid units together with a small portion (about 10%) of the fucose units of the glycoprotein. Another derivative was obtained by treating successively the α_1-acid glycoprotein with a neuraminidase and a β-galactosidase obtained from culture fluids of *Diplococcus pneumoniae* (Hughes and Jeanloz, 1964a). The enzyme treatment removed 100% of the sialic acid units and 80% of the galactose units of the starting material. Both derivatives were shown to be homogeneous by ultracentrifugation analysis and by paper electrophoresis in 0.1 M barbiturate–citrate buffer at pH 8.6. No hydrolysis of the peptide bonds by either acid or enzymic treatment was detected with the ninhydrin reagent.

Dialysis. Dialysis was carried out at 4° against several changes of distilled water in Visking cellophane tubing. The tubing was boiled for 10 min in water before use and then washed thoroughly with distilled water. Examination of the washes showed that a considerable amount of glycerol was present which could not be completely removed, even after exhaustive washing. Since glycerol is also an oxidation fragment produced by the periodate oxidation and subsequent reduction of many carbohydrates, it is important to keep this point in mind when examining fractions for complete cleavage of acetal linkages after treatment with dilute sulfuric acid.

Analyses of Sugar Components. The sugar components were determined according to Hughes and Jeanloz (1964a).

Paper Chromatography. Descending chromatograms on Whatman No. 1 paper were run in the following solvent systems: pyridine–ethyl acetate–acetic acid–water (5:5:1:3) as described by Fischer and Nebel (1955), and the upper phase of 1-butanol–ethanol–water (4:1:5). Sugars, sugar alcohols, and oxidation fragments were detected with the silver nitrate reagent (Trevelyan *et al.*, 1950).

Periodate Oxidation of α_1-Acid Glycoprotein and Derivatives. The α_1-acid glycoprotein and derivatives (0.1–0.5 g, 10 mg/ml) were oxidized for up to 72 hr with sodium metaperiodate (*ca.* 500 moles/mole of glycoprotein) in unbuffered solution at 2–4° in the dark. The consumption of oxidant was estimated by arsenite titration according to the method of Fleury and Lange (Guthrie, 1962). At the end of the reaction, the excess periodate was removed by addition of barium carbonate to pH 7. After stirring for 15 min at room temperature the suspension was centrifuged. The precipitate was washed several times with water, and the washings were combined with the first supernatant which had a brown–yellow color. For large volumes it was found more convenient to filter the neutralized suspension through Whatman No. 50 filter paper.

Reduction and Partial Acid Hydrolysis of Oxidized Glycoproteins. The oxidized glycoprotein was reduced by sodium borohydride (50 mg) at room temperature for 12–16 hr, after which time the solution was dialyzed extensively against distilled water. Then, 2 N sulfuric acid was added to give a final acid concentration of 0.1 N, and the solution (about 0.1–1 l.) was kept at room temperature for 18 hr and dialyzed against three changes of distilled water (1 l. each). The nondialyzable and dialyzable fractions were analyzed or further degraded as required. Aliquots of the dialyzable and nondialyzable fractions were lyophilized for analyses of the sugar compounds remaining and to estimate the weights of material recovered in the two fractions. Control experiments showed that the treatment with sodium borohydride, followed by dilute acid, without prior oxidation, causes no detectable degradation of α_1-acid glycoprotein.

Results

Sequential Periodate Oxidation of Sialic Acid Free α_1-Acid Glycoprotein. The consumption of oxidant by sialic acid free α_1-acid glycoprotein was extremely rapid in the first hour and thereafter rose gradually during the remainder of the reaction. Extrapolation of the second phase of the curve to zero time of oxidation gave a value of approximately 52 moles of oxidant consumed per mole of glycoprotein during the rapid stage of the reaction. The reaction was stopped after 72 hr, and the isolated glycoprotein derivative was reduced with sodium borohydride and subjected to mild acid hydrolysis in 0.1 N sulfuric acid at room temperature for 18 hr. Dialysis of the solution gave a large nondialyzable fraction P_1 and a low molecular weight dialyzable fraction. Aliquots of the two fractions were dried and analyzed for remaining unoxidized monosaccharide components; the results were reproducible for several separate experiments. In the nondialyzable fraction P_1 approximately three-quarters of the total 2-acetamido-2-deoxyglucose residues of the sialic acid free α_1-acid glycoprotein was recovered. In addition P_1 contained 27% of the mannose and a trace (less than 5%) of the galactose units of the starting material (Table I).

No smaller oxidation fragments, glycerol, or glyceraldehyde were found after complete acid hydrolysis, indicating that complete cleavage of the acetal linkages had been achieved. Analysis of the dialyzable fraction obtained after mild hydrolysis in 0.1 N sulfuric acid showed the presence of 5–7% of the original 2-acetamido-2-deoxyglucose units, but no galactose or mannose was detected. After hydrolysis of the dialyzable fraction in 1 N sulfuric acid at 100° for 2 hr, paper chromatography of the neutralized hydrolysate revealed the presence of oxidation fragments, glycerol and glyceraldehyde. No four-carbon fragments, erythritol or threitol, were found.

TABLE I: Sequential Periodate Oxidation of Sialic Acid Free α_1-Acid Glycoprotein.[a]

No. of Oxidations	Nondialyzable Fraction	Monosaccharide Remaining in Nondialyzable Fraction							
		Fucose		2-Acetamido-2-deoxyglucose		Galactose		Mannose	
		(Moles[b])	(%)	(Moles[b])	(%)	(Moles[b])	(%)	(Moles[b])	(%)
0		3	100	27	100	18	100	14	100
1	P_1	0	0	19.4	72	0.9	5	3.7	27
2	P_2			12.7	47	0	0	3.7	27
3	P_3			4.7	18			0	0
4	P_4			0.8	3				

[a] The glycoprotein was obtained by removal of sialic acid units by mild acid hydrolysis. The derivative was subjected to several sequential periodate oxidations as discussed in the text. Each step involved oxidation for 72 hr at 2–4° with sodium metaperiodate followed by sodium borohydride reduction and mild acid hydrolysis. After dialysis, the nondialyzable fraction was isolated after each step and analyzed as described in Materials and Methods. The molar concentration of the original glycoprotein derivative was determined by absorption at 280 mμ. [b] Per mole of glycoprotein.

Periodate Oxidation of Fraction P_1. Periodate oxidation of P_1 was carried out as described for the sialic acid free glycoprotein, and the oxidized product was treated with sodium borohydride. The oxidized, then reduced, derivative was hydrolyzed in dilute sulfuric acid and the hydrolysate was dialyzed against several changes of distilled water. The nondialyzable fraction, P_2, and the dialyzable low-molecular weight fraction so obtained were analyzed for intact monosaccharides remaining after the second degradation. In the oxidation of P_1, the small amount of the galactose units of this derivative was completely destroyed, together with about 30–40% of the hexosamine of this fraction (Table I). The mannose content of P_2 remained identical with that of P_1. The molar ratio of 2-acetamido-2-deoxyglucose to mannose in P_2 was almost equivalent to 3:1 (Table I). Analysis of the dialyzable fraction showed the absence of intact monosaccharides, although traces of oxidation fragments were present.

Periodate Oxidation of P_2. As shown in Table I, degradation of P_2 gave a nondialyzable product, P_3, in which the only stoichometrically significant monosaccharide unit present was 2-acetamido-2-deoxyglucose. Analyses of this fraction obtained in several separate experiments for galactose and for mannose showed in some cases the presence of traces of these sugars.

One sample of P_3 was subjected to a fourth degradative sequence, and the resulting nondialyzable fraction P_4 was analyzed for remaining hexosamine (Table I). As shown by the Elson–Morgan reaction (Gardell, 1953), after hydrolysis with 6 N hydrochloric acid approximately 85% of the amino sugar of P_3 had been destroyed. Thus 97% of the 2-acetamido-2-deoxyglucose residues of the sialic acid free glycoprotein derivative was destroyed after four sequential degradations.

The possibility was examined that some of the 2-acetamido-2-deoxy-D-glucose residues of the nondialyzable fraction obtained after three sequential degradations of the sialic acid free α_1-acid glycoprotein were joined in β-glycosidic linkage either with other monosaccharide units or with hydroxyl-containing amino acids present in the polypeptide moiety. The glycoprotein derivative was incubated for 48 hr at pH 5.3 and 37° at a concentration of 5 mg/ml with purified β-*N*-acetylglucosaminidase of *D. pneumoniae* (Hughes and Jeanloz, 1964b). No release of 2-acetamido-2-deoxyglucose was detected by the Morgan and Elson reaction (Gardell, 1953). It has been previously shown (Hughes and Jeanloz, 1964b) that the enzyme does not participate in transfer reactions at the level of substrate concentration used in these experiments. The results indicate the probable absence of β-glycosidically linked 2-acetamido-2-deoxy-D-glucose residues in P_3. Under similar conditions, the purified enzyme readily releases 2-acetamido-2-deoxyglucose units from a derivative of the α_1-acid glycoprotein obtained by successive treatment of the macromolecule with highly purified neuraminidase and β-galactosidase isolated from *D. pneumoniae* (Hughes and Jeanloz, 1964b).

Sequential Periodate Oxidation of Neuraminidase and β-Galactosidase Degraded α_1-Acid Glycoprotein. The derivative obtained by successive treatments of α_1-acid glycoprotein with neuraminidase and β-galactosidase of *D. pneumoniae* was submitted to sequential periodate oxidation in the manner used for sialic acid free α_1-acid glycoprotein. The results are shown in Table II. The periodate oxidations were carried out at 2–4° for 6 hr only, in order to reduce the slow "overoxidation" phase of the reaction which would lead to more ex-

TABLE II: Sequential Periodate Oxidation of Enzymically Treated α_1-Acid Glycoprotein.[a]

No. of Oxidations	Nondialyzable Fraction	Monosaccharide Remaining in Nondialyzable Fraction							
		Fucose		2-Acetamido-2-deoxyglucose		Galactose		Mannose	
		(Moles[b])	(%)	(Moles[b])	(%)	(Moles[b])	(%)	(Moles[b])	(%)
0		3	100	27	100	4	100	14	100
1	P_1	0	0	16	60	0.2	5	5.7	61
2	P_2			5.4	20	0	0	5.5	39
3	P_3			5.8	22			1.0	7

[a] The glycoprotein was treated successively with purified *Diplococcus pneumoniae* neuraminidase and β-galactosidase. The sequential periodate oxidation was performed as described in the text. Each step involved oxidation for 6 hr at 2–4° with sodium metaperiodate followed by sodium borohydride reduction and mild acid hydrolysis. The nondialyzable fraction after each step was isolated and analyzed as described in Materials and Methods. The molar concentration of the original glycoprotein derivative was determined by absorption at 280 mμ. [b] Per mole of glycoprotein.

tensive destruction of the monosaccharide and amino acid components. After each oxidation the sample was reduced with sodium borohydride, then subjected to mild acid hydrolysis, and the nondialyzable fraction was isolated as previously described. The first degradation of the glycoprotein derivative removed about 40% of the hexosamine and mannose units of the derivative (Table II). A trace of galactose was found in the acid hydrolysate of P_1. The fucose units were completely destroyed. A second degradation decreased the hexosamine content in the nondialyzable fraction P_2 to 20% of the original hexosamine content of the glycoprotein derivative (Table II). The mannose component was partially resistant to this second degradation. A third treatment of the glycoprotein removed essentially all of the mannose units of P_2, leaving 2-acetamido-2-deoxyglucose as the only significant monosaccharide remaining in the nondialyzable fraction P_3 (Table II). The amount of hexosamine in P_3 was about 22% of the original hexosamine content of the starting material and was similar to that obtained after three sequential periodate oxidation of sialic acid free α_1-acid glycoprotein (Table I).

Discussion

The values for per cent destruction of hexosamine, galactose, mannose, and fucose of the sialic acid free α_1-acid glycoprotein after oxidation for 72 hr are in good agreement with those reported earlier (Eylar and Jeanloz, 1962b) using a shorter oxidation time. The oxidation was carried out with a sufficiently large excess of periodate, and therefore the apparent resistance to oxidation of part (5%) of the galactose residues of sialic acid free α_1-acid glycoprotein is not due to an exhaustion of oxidant. The stability of oxidation may be due in part to protection by the L-fucose residues. If one assumes that all of the fucosyl units are linked to position 3 of the galactose residues, up to 15% of the total galactose units of sialic acid free glycoprotein might be unoxidized by periodate. Methylation of sialic acid free α_1-acid glycoprotein resulted in the isolation in acid hydrolysates of 2,4,6-tri-*O*-methyl-D-galactose as well as 2,3,4,6-tetra-*O*-methyl-D-galactose (Jeanloz and Closse, 1963). These results are consistent with a 3-substituted galactose unit being present internally in the carbohydrate chains.

About eight 2-amino-2-deoxyglucose units (28%) per mole of sialic acid free α_1-acid glycoprotein were removed by the first periodate oxidation, but two units (5–7%) per mole only were found in the dialyzable fraction obtained after reduction and mild acid hydrolysis. The latter units were released probably by the oxidation of a mannose unit or, less likely, of another 2-amino-2-deoxyglucose unit to which they were glycosidically linked. Thus probably about five–six (or 40%) of the 2-amino-2-deoxyglucose units located in the third position from the sialic acid end of the carbohydrate chains are linked through C-6 to the galactosyl residues. No 2-amino-2-deoxy-6-*O*-(β-D-galactosyl)-D-glucose disaccharide has been isolated from the acid hydrolysate of the α_1-acid glycoprotein nor any 2-amino-2-deoxy-3,4-di-*O*-methyl-D-glucose from the hydrolysate of the methylated α_1-acid glycoprotein, but such a disaccharide has been found to be a receptor for the enzymatic addition of sialic acid (Jourdian *et al.*, 1963). The fact that no erythrose has been found in the dialyzable fraction obtained after one degradation shows unequivocally that the oxidized mannose units are linked at C-2 or C-6, but not at C-4.

A second degradation of the sialic acid free α_1-acid glycoprotein resulted in the reduction of the hexosamine content of P_1, indicating the presence of 2-acetamido-2-deoxyglucose as nonreducing end groups in P_1. The mannose units resistant to oxidation are probably situated more internally in the carbohydrate chains, most probably as branch points, since the oxidation of seven 2-amino-2-deoxyglucose units (25%) per mole of

glycoprotein exposes four mannose units (27%) and eight 2-amino-2-deoxyglucose units (29%) to further oxidation.

The product of three sequential degradations of sialic acid free α_1-acid glycoprotein contains only hexosamine, which clearly implicates for the first time the involvement of 2-acetamido-2-deoxyglucose in the linkage between the carbohydrate chains and the protein moiety of the glycoprotein. From the content of 2-acetamido-2-deoxyglucose in the nondialyzable fractions P_3 (Tables I and II) and the starting weight of the glycoproteins, it may be calculated that there are 5–6 carbohydrate–protein linkages in the intact α_1-acid glycoprotein. The experimental value can be only a minimal one since losses of material undoubtedly occurred during the extensive practical manipulations required for the over-all degradation. It is also possible that some chains would be destroyed more rapidly than other more highly branched structures. Studies of glycopeptide fractions (Kamiyama and Schmid, 1962a,b; Eylar, 1962; Izumi *et al.*, 1961, 1962; Bourrillon *et al.*, 1964) isolated from α_1-acid glycoprotein after exhaustive proteolytic attack have suggested that the amino acid involved in the protein–carbohydrate link is aspartic acid, probably in the amide form. This finding, taken with the results of the present paper, indicates that the linkage is between 2-acetamido-2-deoxyglucose and aspartic acid (or asparagine) residues. N^2-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-L-asparaginyl structure was demonstrated for the linkage of carbohydrate and polypeptide moieties of ovalbumin (Marks *et al.*, 1963; Marshall and Neuberger, 1964).

The nondialyzable fraction P_2 remaining after two degradations contained only hexosamine and mannose in the approximate molar ratio 3:1 (Table I). The segments of the carbohydrate chains in closest proximity to the protein moiety of the α_1-acid glycoprotein therefore appear to consist of sequences with a similar carbohydrate composition as the polysaccharide prosthetic group of ovalbumin and ribonuclease B (Clamp and Hough, 1963; Plummer and Hirs, 1963).

The results obtained with the sialic acid free glycoprotein were essentially supported by the sequential periodate oxidation of a derivative obtained by treatment of the α_1-acid glycoprotein successively with *D. pneumoniae* neuraminidase and β-galactosidase (Table II). The nondialyzable fraction P_3 remaining after three degradations of the enzymically treated glycoprotein also contained 2-acetamido-2-deoxyglucose as the monosaccharide remaining in the largest amount (Table II).

The fact that each of the various polysaccharide units attached to the polypeptide core of the glycoprotein may have a different chain length, a different composition, and a different structure limits severely the interpretation of data obtained from the study of the whole molecule. Certain structures, however, may be deduced: about 8–9 chains are composed of *O*-β-D-galactopyranosyl-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2- or -6)-D-mannopyranosyl residues. The remaining five chains are composed of *O*-β-D-galactopyranosyl-(1→6)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2- or -6)-D-mannosyl residues. Approximately 30–40% of the chains possess branch points at C-3 or C-4 of the mannosyl residues; each mannose unit is substituted probably with two 2-acetamido-2-deoxyglucose units.

These structures do not disagree with the results of the acid degradation (Eylar and Jeanloz, 1962a) but they have not as yet been confirmed by the methylation technique (A. Closse, E. H. Eylar, N. R. Williams, and R. W. Jeanloz, unpublished). An additional conclusion to be deduced from the sequential periodate oxidation of the α_1-acid glycoprotein is the linkage of some 2-amino-2-deoxyglucose units to other 2-amino-2-deoxyglucose units, possibly as branch points.

Attempts were made to subject intact α_1-acid glycoprotein to sequential periodate oxidation. In this case, however, a considerable carbohydrate content including galactose and mannose, as well as 2-acetamido-2-deoxyglucose, remained after five sequential degradations. The nondialyzable fraction obtained after a single degradation of the intact α_1-acid glycoprotein was examined by ultracentrifuge analysis and showed a single homogeneous peak with a sedimentation constant higher than that found for the starting material. Oxidation between C-7 and C-8 of the aliphatic chain of the *N*-acetylneuraminyl residue produces one aldehyde group per each sialic acid unit. This aldehyde group may react with other carbohydrate units to form intermolecular links, giving rise to molecular complexes which would prevent further oxidation of the carbohydrate chains. Similar results have been reported by Barker and Whitehead (1963). This type of condensation is not likely to happen with the aldehyde groups produced by the oxidation of the galactose, mannose, and 2-acetamido-2-deoxyglucose pyranose units, for it has been shown that the dialdehydes produced by cyclic structures form cyclic hemialdal or cyclic internal semiacetal structures (Guthrie, 1961).

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References

Barker, S. A., and Whitehead, P. H. (1963), *Clin. Chim. Acta 8*, 848.

Bourrillon, R., Got, R., and Meyer, D. (1964), *Biochim. Biophys. Acta 83*, 178.

Clamp, J. R., and Hough, L. (1963), *Chem. Ind. (London)*, 82.

Eylar, E. H. (1962), *Biochem. Biophys. Res. Commun. 8*, 195.
Eylar, E. H., and Jeanloz, R. W. (1962a), *J. Biol. Chem. 237*, 622.
Eylar, E. H., and Jeanloz, R. W. (1962b), *J. Biol. Chem. 237*, 1021.
Fischer, F. G., and Nebel, H. J. (1955), *Z. Physiol. Chem. 302*, 10.
Gardell, S. (1953), *Methods Biochem. Analy. 6*, 314.
Goldstein, I. J., Hay, G. W., Lewis, R. A., and Smith, F. (1959), Abstracts, 135th National Meeting of the American Chemical Society, Boston, Mass., p. 3D.
Guthrie, R. D. (1961), *Advan. Carbohydrate Chem. 16*, 105.
Guthrie, R. D. (1962), *Methods Carbohydrate Chem. 1*, 435.
Hughes, R. C., and Jeanloz, R. W. (1964a), *Biochemistry 3*, 1535.
Hughes, R. C., and Jeanloz, R. W. (1964b), *Biochemistry 3*, 1543.
Hughes, R. C., and Jeanloz, R. W. (1964c), Abstracts, 1st. Meeting Fed. Europ. Biochem. Soc., London, p. 63.
Izumi, K., Makino, M., and Yamashina, I. (1961), *Biochim. Biophys. Acta 50*, 196.
Izumi, K., Makino, M., and Yamashina, I. (1962), *J. Biochem. (Tokyo) 51*, 365.
Jeanloz, R. W., and Closse, A. (1963), *Federation Proc. 22*, 538.
Jourdian, G. W., Carlson, D., and Roseman, S. (1963), *Federation Proc. 22*, 464.
Kamiyama, S., and Schmid, K. (1962a), *Biochim. Biophys. Acta 58*, 80.
Kamiyama, S., and Schmid, K. (1962b), *Biochim. Biophys. Acta 63*, 266.
Marks, G. S., Marshall, R. D., and Neuberger, A. (1963), *Biochem. J. 87*, 274.
Marshall, R. D., and Neuberger, A. (1964), *Biochemistry 3*, 1596.
Plummer, T. H., and Hirs, C. H. W. (1963), *J. Biol. Chem. 238*, 1396.
Schmid, K. (1950), *J. Am. Chem. Soc. 72*, 2816.
Schmid, K. (1953), *J. Am. Chem. Soc. 75*, 60.
Smith, F., and Unrau, A. M. (1959), *Chem. Ind. (London)*, 881.
Trevelyan, W. E., Proctor, D. P., and Harrison, J. S. (1950), *Nature 166*, 444.
Weimer, H. E., Mehl, J. W., and Winzler, R. J. (1950), *J. Biol. Chem. 185*, 561.
Whelan, W. J. (1960), *Ann. Rev. Biochem. 29*, 105.