

ported by Schmid et al. (474, 475). Recently the amino acid sequence of the polypeptide chain of AGP was deduced from the cDNA nucleotide sequence (64). There was an excellent agreement with the amino acid sequence reported earlier by Schmid et al. (475). There was only a difference at four places on the polypeptide chain (64, 469, 475).

The five carbohydrate moieties of AGP are located in the first half of the peptide chain and are linked to asparagine residues. The carbohydrate moieties consist of about 11% sialic acid, 14% neutral hexoses, 14% hexosamine, and 1% fructose (512). It should be pointed out here that human plasma proteins contain only N-acetylneuraminic acid, whereas proteins of other species may have variable proportions of the N-acetyl and N-glycosyl derivatives (382, 512, 577). The sialic acid residues, being easily removable (224), may be linked to C-2, C-3, C-4, or C-6 of the galactose residues (258, 469). The unusually low isoionic point of 3.4 is caused by the high sialic acid content (257, 469, 557). The literature up to 1972 dealing with the chemical identification of each of the five different carbohydrate moieties of AGP is reviewed by Jeanloz (257). The structure of the carbohydrate moiety of AGP has been studied extensively in recent years (25, 167, 224, 296, 477, 486, 487, 590). Fournet et al. (167) determined the primary structures of 16 asialo carbohydrate units derived from AGP, using 360 MHz proton nuclear magnetic resonance (NMR) spectroscopy. The asialocarbohydrate units can be grouped in five classes with bi-, tri-, and tetraantennary structures, respectively, for the first three classes. The fourth and fifth classes have also a tri- or a tetraantennary structure, but with an additional fucose residue. In addition to the five chains reported by Fournet et al. (167), Yoshima et al. (590) elucidated the structure of three new sugar chains. Hansen et al. (215) reported recently significant differences in antennary structure of the glycan part of AGP from different patient groups.

Recently, Cardon et al. (102) succeeded in analyzing the sialyloligosaccharides of AGP by high-performance liquid chromatography. They found that native AGP contains no traces of neutral oligosaccharides, but only monosialylated (5.8%), disialylated (34.6%), trisialylated (43.3%), and tetrasialylated (16.2%) glycans.

Crystals of AGP have been described (344, 345, 467). However, a detailed three-dimensional structure as determined from X-ray crystallography has not yet been reported. Schmid et al. (473) studied the tertiary structure of AGP in solution using circular dichroism (259) and chemical modification methods.

Aubert and Loucheux-Lefebvre (25) reported that the protein moiety of AGP contains 21% alpha-helix, 21% beta-sheet, 8 reverse beta-turns, and 40% unordered structure. They observed that, of the five carbohydrate moieties, four are linked to asparagine residues which are situated either in a reverse beta-turn or in regions where charged and polar residues are numerous, that is,

on the outside of the protein. They also reported that the carbohydrate moieties do not produce any perturbation of the protein conformation. Schmid et al. (472) reported that, even after removal of 85% of the carbohydrate content, the secondary structure of the AGP was not affected.

Several studies show that the physical-chemical properties of AGP can change during disease states. Recently, Chandrasekaran et al. (104) isolated from liver metastases of lung, colon, and breast tumors two variants of AGP with common immunological determinants and almost identical amino acid compositions but different amounts of carbohydrate. Rudman et al. (448) found an abnormal AGP in the plasma of patients with neoplastic disease. This abnormal AGP had a molecular weight of between 40,000 and 50,000, a normal protein moiety, but multiple abnormalities in the carbohydrate moiety. An AGP variant in the plasma of cancer patients has been reported (162, 595-598). This abnormal AGP contains a chromophoric group which has the characteristics of a pteridine. It has a less negative charge, although its sialic acid content is not reduced. It exists partially in a polymeric form, possibly due to the cross-linking effect of the chromophoric group. Its extinction values and optical rotary dispersion (ORD) data indicate differences in the secondary structure. Ziegler et al. (596, 597) reported recently that the decreased negative charge of this AGP (595) is due to the binding of the pteridine chromophore to the sialic acid antennae. Serbource-Goguel et al. (489, 490) reported the presence of partially desialylated AGP in plasma from patients with liver disease. The degree of desialylation of AGP was dependent on the severity of the liver disease.

D. Microheterogeneity of Alpha-1-acid Glycoprotein

The several heterogeneous forms or variants of AGP (table 2) can have different electrophoretic patterns. This phenomenon is called the microheterogeneity of AGP.

The results of the studies on the microheterogeneity of the variants of AGP are summarized in table 4. From this table it follows that the microheterogeneity is dependent on the state of AGP (native or asialo), the characterization technique used, and the origin of the AGP preparation (from normal volunteers or patients; 14, 62, 73, 74). Umetsu et al. (543) introduced recently a new technique for isoelectric focusing, which is not included in table 4. Hanssen et al. (215) reported recently that the electrophoretic microheterogeneity of AGP can be evaluated in terms of the antennary structure of the glycan part of AGP and that significant differences in glycan structure were found in different patient groups. Serbource-Goguel et al. (489, 490) reported recently about the alterations in relative proportions of microheterogeneous forms of AGP in liver disease. Mackiewicz et al. (323) observed that the microheterogeneous forms can be used as indicators of rheumatoid arthritis activity.

Charge differences in the polypeptide and carbohy-

drate chains and structural differences in the carbohydrate moiety of AGP also play a role in the observed microheterogeneity: differences in the polypeptide chain are determined genetically, whereas differences in the carbohydrate moiety are dependent on the severity of the disease (table 4).

Tinguely et al. (529) reported that the S-variant (see table 4, no. VI) of AGP has a somewhat stronger affinity for amitriptyline and nortriptyline (table 8, nos. XXXII and III). Up till now, no other studies have been reported on the effect of the microheterogeneity of AGP on the pharmacokinetics of the drug binding (15).

III. Biological Functions of Alpha-1-acid Glycoprotein

A. Alpha-1-acid Glycoprotein as Acute Phase Protein

Since the sixties it has become clear that AGP is a plasma protein, the level of which can vary considerably during several physiological and pathological conditions. Tables 5 and 6 give a survey of these conditions. The variations in the AGP level proved to be dependent on the severity of the disease states. Whereas for healthy people plasma levels of AGP are reported to range between about 40 and 110 mg/100 ml, AGP values of up to about 300 mg/100 ml have been found during diseases (396, 406, 426, 513, 538). In order to substantiate these conclusions, data were collected from the literature. Table 6 gives a survey of quantitative data on AGP and HSA levels in the plasma of healthy people and of patients with various diseases.

From the data in table 6, it follows that the normal value of the average HSA concentration in plasma of about 4 g/100 ml can decrease until about 2 g/100 ml during disease. It further follows that the normal average plasma levels of AGP are between 50 and 100 mg/100 ml; 65% of the normal cases have a level between 60 and 80 mg/100 ml; the average value is 73 mg/100 ml. The table also gives data concerning the increase in AGP concentration in acute phase situations. About 50% of the data represent a situation in which the average value in the acute phase is twice as high as the average value in the normal situation. About 35% of the data give values that are 3 times as high. It can therefore be concluded that both AGP and HSA can be classified among the acute phase proteins (12, 293) and that especially the level of AGP in plasma can be used as diagnostic and prognostic aid during the treatment of several diseases (12, 164, 165, 178, 184, 216, 218, 221, 333, 351, 392, 423, 434, 496, 533, 541, 553, 554, 562, 595). In this study, only the acute phase behavior of AGP will be discussed extensively. The observed increased AGP level and decreased HSA level are also important in relation to drug binding; this will be discussed in section IV.

Other reports are known in which the AGP concentration was measured as a function of time (10, 80, 105, 106, 114, 122, 129, 133, 137, 152, 188, 196, 198, 199, 216, 218, 240, 260, 288, 323, 446, 454, 525, 562, 586). Monitoring

TABLE 6
Survey of several disease states and physiological conditions with varying AGP levels in human plasma

No.	Pathological/physiological condition	Ref.
I	Acidosis	140, 343
II	Age	5, 6, 16, 59, 60, 70, 77, 134, 139, 201, 242, 287, 290, 391, 397, 398; table 6, nos. VII-VII, L, LI
III	Alcohol use	16, 26, 190, 452
IV	Allergy	103, 295, 393
V	(Ventricular) arrhythmia	10a, 161
VI	Arthritis	1, 42-44, 139, 288, 323, 409, 479-481, 524
VII	Bacterial infection in neonatal period	13, 56, 57, 70, 183, 454, 455; table 6, nos. VIII-XII
VIII	Burn	63, 332; table 6, nos. XIV, XV
IX	Cancer (breast, colorectal, lung, ovaries)	7-9, 76, 110-112, 114, 118, 162, 184, 184, 186, 216, 221, 241, 250, 273, 392, 409, 434, 502, 523, 526, 541, 554, 559, 562; table 6, nos. XVI-XXXV
X	Chest pain	80, 169, 269, 400; table 6, no. LXXII
XI	Chronic inactive pyelonephritis	426
XII	Chronic hemodialysis patients	147, 226, 290, 391, 435, 589
XIII	Chronic pain	178; table 6, no. LXXXII
XIV	Chronic renal failure	146, 147, 435
XV	Chronic ulcerative colitis	137
XVI	Crohn's disease	152, 409, 479, 481
XVII	Depression	85, 88, 192, 193, 380, 552; table 6, nos. XLV, XLVI
XVIII	Epilepsy	304, 339, 432, 441, 531; table 6, nos. XLVII-IL
XIX	Genetic factor	16, 59, 60; table 6, no. LI
XX	Gliomas	562
XXI	Hepatitis	391, 489, 490; table 6, nos. LV, LVI
XXII	Hormonal contraceptives use	59, 81, 301, 302, 408, 585; table 6, nos. LXXX, LXXXI
XXIII	Hyperlipoproteinemia	145, 147, 226
XXIV	Hyperlipidemia	99, 153, 154
XXV	Hypertension	200
XXVI	Inflammation	62, 127, 253, 271, 409, 428, 430, 442, 479, 480-482, 488, 561; table 6, nos. LVIII, LXIII
XXVII	Liver cirrhosis	26, 36, 187, 190, 391, 409, 447, 489, 490, 493, 524; table 6, nos. XXXVIII-XLI

TABLE 5—Continued

No.	Pathological/physiological condition	Ref.
XXVIII	Liver carcinoma	114
XXIX	Multiple sclerosis	426
XXX	Myocardial infarction	10, 34, 80, 105, 106, 183, 185, 156, 161, 169, 260, 269, 400, 439, 440, 443, 447, 449, 493, 504, 553, 583; table 6, nos. LXVIII-LXXVII
XXXI	Nephrotic disease	145, 147, 391, 496, 509, 569
XXXII	Obesity	16, 47, 48; table 6, nos. LXXVIII- LXXIX
XXXIII	Pregnancy	70, 117, 128, 135, 163, 204, 218, 235, 238, 265, 357, 367, 403, 404, 427, 454, 455, 521, 586; table 6, nos. LXXXIV XCIII
XXXIV	Renal disease	120, 122, 145, 146, 206, 270, 391, 409, 435, 447, 493, 534; table 6, nos. XCV, XCV
XXXV	Sex	5, 47, 59, 70, 129, 152, 163, 178, 193, 287, 290, 380, 442; table 6, nos. LXXI, LXXXIX
XXXVI	Smoking	46, 59, 134, 241, 287; table 6, nos. XCVII, XCVIII
XXXVII	Stress	156, 178, 534
XXXVIII	Surgery	129, 185, 152, 163, 171, 215, 232, 240, 351, 409, 553
XXXIX	Trauma	129, 153-155; table 6, nos. IC-CVI
XI	Uremic disease	5, 145, 147, 206, 226, table 6, nos. CVII- CXI
XLI	Wound healing	198, 351

AGP levels in this way is a useful aid in clinical therapy (table 6). Most of these data are collected from cancer patients. The elevated level of AGP for these patients (but not as a function of time) has been described extensively (76, 116, 221, 241, 273, 333, 434, 502, 523, 526, 541, 554, 559, 562; table 6, nos. XVI-XXXV). Changes in the AGP level have been correlated with the response of cancer patients to chemotherapy treatment (164, 184, 221, 591).

After myocardial infarction, higher levels of AGP (Table 6, nos. LXVII-LXXVII) with peak values on days 4 to 5 have been reported (10, 260), although Voulgari et al. (553) could not detect an appreciable change in the AGP level during the first 10 days after myocardial infarction. Other reports substantiate the use of AGP levels for diagnostic and prognostic purposes in this field (80, 105, 106, 504).

Rises in the level of AGP (for survey, see table 6) have also been observed after surgery (129, 152, 163, 351, 553), in inflammation (10, 137, 351), and during infections (56, 57, 117, 199, 279, 405, 453-455, 553). Other applications have been described in patients with chronic pain (178), rheumatoid arthritis (323), hepatic diseases (114), multiple sclerosis (426), renal dysfunction (146), and during wound healing (198, 351).

AGP levels were also monitored during pregnancy (table 6, nos. LXXXIV-XCIII). A decrease depending on the stage of gestation was observed (57, 70, 196, 236, 506, 521). Levels of AGP have been reported to be higher in the first and third trimester with a decline around 24 wk gestation (218). The use of contraceptive steroids also decreases the AGP level (59, 81, 301, 302, 408, 586; table 6, nos. LXXX-LXXXI).

Lower AGP levels were observed in the serum of patients with liver cirrhosis (28, 36, 187, 190, 391, 409, 447, 489, 490, 493, 524; table 6, nos. XXXIX-XLI) and in the serum of newborns (13, 57, 70, 183, 199, 284, 404, 423, 554, 555, 585; table 6, nos. IX-XII, LXXXVII-XCIII). The occurrence of lower AGP levels in serum of newborns (more than 2 times less than the value in healthy adults) can explain the complications that occur directly after delivery in a mother using a drug therapy.

It would be interesting to discover the reasons for this changing level of AGP. Winzler and Bocci (578) reviewed the turnover of the major plasma glycoproteins. They reported that most of the circulating plasma glycoproteins, including AGP, are synthesized in the liver (51, 127, 262, 352, 355, 456, 580), probably in the form of an intrahepatic precursor (366). Weisman et al. (561) studied the turnover of AGP in man and observed an increase in synthesis in patients with inflammatory disease. Several reports in which the mechanism of the synthesis of AGP was studied using a perfused rat liver or cultures of rat hepatocytes (38, 144) point to the role of increased mRNA in this context. Diarra-Mehrpour et al. (144) recently reported that, after a high dose of 17-alpha-ethynylestradiol and after acute inflammation, rats showed an increase in the plasma concentration of AGP, due to hepatic accumulation of the AGP-mRNA. They further concluded that different mechanisms and/or pathways are probably involved in regulating the synthesis of AGP under various stimuli such as glucocorticoids. Similar conclusions were reached by other investigators (141, 291, 312, 428, 430, 547).

The homology with immunoglobulins is also stressed. Ikenaka et al. (248) reported that AGP was the first single-chain protein that was found to show sequence similarity with haptoglobin and particularly with the immunoglobulins (36% and 75%, respectively). Schmid et al. also observed the homology of AGP with the immunoglobulins (474, 475).

Toh et al. (532) reported recently that membrane AGP has some structural homologies with the β -chain of HLA-DC, with immunoglobulin, and with the epidermal