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Macroamylasemia and Other Immunoglobulin-Complexed Enzyme Disorders

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Macroamylase is a circulating complex of immunoglobulin linked to normal amylase in most cases. Its physical properties are heterogeneous, but its large size impairs renal filtration. Macroamylasemia usually causes hyperamylasemia and an amylase clearance:creatinine clearance (C_{AM}:C_{CR}) ratio of less than 1 percent. Macroamylasemia occurs in 2.5 percent of hyperamylasemic patients, and 1 percent of apparently healthy subjects with normal amylase levels. It often accompanies diseases of aberrant immunity or conditions in which pancreatitis must be ruled out. This disorder should be considered in a patient with asymptomatic hyperamylasemia because its detection can obviate a prolonged diagnostic workup. The condition requires no treatment and may be transient. Macroamylasemia is one of several immunoglobulin-complexed enzyme (ICE) disorders. MacroLDemia, an ICE disorder of lactate dehydrogenase (LD), shares features with macroamylasemia. These and other ICE disorders appear to represent nonspecific dysproteinemic responses to disease.

A PATIENT WITH unexplained hyperamylasemia is often subjected to numerous and expensive blood tests, procedures or even laparotomy. A frequently overlooked cause of hyperamylasemia is macroamylasemia, although macroamylase can be assayed in ten minutes.[1] Macroamylase, a macromolecular complex with amylase activity, whose size prevents renal filtration and clearance, occurs in 2.5 percent of patients with hyperamylasemia and 1 percent of the general population. Its presence was first discovered by Wilding and coworkers[2] in 1964 and designated "macroamylasemia" by Berk and colleagues[3] in 1967.

Macroamylasemia involves an immunoglobulin-amylase complex in most cases and possibly a polysaccharide-amylase complex in a few others. It can occur transiently during acute illness or chronically during prolonged illness, and may be present even when serum amylase activity is within normal limits. Macroamylasemia may reflect disease-induced dysproteinemia or may represent an early marker of systemic disease in some patients. The discovery of this disorder has

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ABBREVIATIONS USED IN TEXT

$C_{AM}:C_{CR}$ = amylase clearance:creatinine clearance (ratio)
CK = creatine kinase
EDTA = ethylenediaminetetraäcetate
HES = hydroxyethyl starch
ICE = immunoglobulin-complexed enzyme (disorder)
LD = lactate dehydrogenase
MacroLD = macromolecular lactate dehydrogenase
MW = molecular weight

generated new questions about the human immune system and the body's response to disease. Because of recent advances in the understanding of macroamylasemia and other less frequent immunoglobulin-complexed enzyme disorders, these conditions are reviewed below.

Macroamylasemia

Physical Properties of the Macroamylase Complex

Macroamylase is heterogeneous in its physical properties. It can be clearly distinguished from normal amylase by chromatography, ultracentrifugation and electrophoresis. Normal serum amylase can be dissociated from the macroamylase complex in some cases through acidification.

Chromatography

Normal serum amylase has a molecular weight (MW) of 45,000[4,5] and elutes from a Sephadex G-200 gel column with cytochrome C, whose MW is 13,000. The sedimentation coefficient (which is roughly proportional to MW)[6] of cytochrome C is 4.5 Svedberg units (S).[1] Macroamylase complexes have been recovered from Sephadex G-200 at all positions from 7S to 19S.[1,3,7-31] With Sephadex G-100, macroamylases generally emerge at or near the void volume.[1,2,21,32-37] Dextran gels are inadequate to estimate the MW of a macroamylase complex because macroamylase interacts with the gel, partially dissociating into smaller complexes, plus free amylase.[1,13,15,16,20,21,38] Gel permeation chromatography of free amylase at 4°C on dextran appeared to lead to formation of macroamylase in one study[13] but not in another.[16] Polyacrylamide gel has been used for chromatography,[11,13,15,38-44] but may be more difficult to use than dextran.[15] Agarose gel has also been used successfully.[20] Gel filtration in a reducing solvent has been employed to estimate molecular weights of protein better by eliminating molecular interactions.[45] This procedure, however, partially[29] or completely[20] eliminates the amylase activity of macroamylase, which complicates subsequent assays.

Ultracentrifugation

Ultracentrifugation of human serum can purify macroamylase complexes.* Normal serum amylase has a sedimentation coefficient of 4.5S, and macroamylase complexes have been identified at all positions from 7S to 19S by this method, with most of them at 7S or 11S. In one series,[16] 4 of 22 patients had macroamylase complexes with sedimentation patterns too broad to be assigned a specific S value. In each patient's serum, there may have been a family of macroamylases, each with its own S value.

Electrophoresis

Macroamylase can be distinguished from normal serum amylase using electrophoresis,† but it lacks a specific migration pattern. Normal serum amylase can be fractionated electrophoretically into a slowly migrating and a quickly migrating component[27,48,51]; the former corresponds in position to the amylase in pancreatic extracts, while the latter coincides in position with parotid gland amylase. Macroamylase migrates differently from both of these isoenzymes when using an appropriate matrix such as cellulose acetate,[33,36,39,49,50] polyacrylamide,[3,29,48] or agar,[27,28,33] but not paper (32 of 33 macroamylasemic sera tested).[2,3,10,52] Macroamylase has been observed to migrate both slower[2,3,27-29,33,36,41,48] and faster[27,39,49,50] than pancreatic amylase. At least seven salivary and three pancreatic isoamylases have been distinguished electrophoretically.[53] Additional amylases can arise during diseases that affect the liver, lungs and genital tract,[54] although it is not clear whether these represent posttranslational modifications of the two basic gene products or additional genes.[54-56] Thus, the presence of an "abnormal" electrophoretic band with amylase activity cannot necessarily be assumed to represent macroamylase, especially when the electrophoretic behavior of amylase is so variable.

Dissociation

Specific antigen-antibody reactions may dissociate in acid.[57] Macroamylase complexes have been

*References 7, 10, 13, 15, 16, 20, 22, 46, 47.
†References 2, 3, 27-29, 33, 36, 39, 41, 48-50.

dissociated by acid into amylase monomers with normal properties in most,* but not all[13,15,29,38,60] cases tested. The kinetics of dissociated amylase are indistinguishable from normal amylase.[7,11] Susceptibility to acid dissociation may be inversely proportional to complex size.[15] Dissociation varies in alkali[50,61] and is complete in urea.[11] In 30 percent ammonium sulfate [$(NH_4)_2SO_4$] macroamylase and gamma globulins are insoluble, whereas normal amylase is soluble.[20] *Bacillus subtilis* contains a macroamylase, consisting of divalent ion-linked amylase monomers, that is reduced to monomers by the chelating agent ethylenediaminetetraäcetate (EDTA)[62]; human macroamylase is not dissociated by EDTA,[7,8,10,11] and no experiment has shown polymerization of human amylase.

Nonspecific covalent disulfide bonds can form between globulins and circulating proteins in patients with paraproteinemias.[63] Mercaptoethanol dissociates covalent bonds and can dissociate 11S polymeric immunoglobulins to 7S monomers.[64] The 11S macroamylase in one patient was converted completely to a 7S macroamylase by mercaptoethanol.[7] Other experiments with this reducing agent have been inconclusive.[11] There is no evidence for covalent binding of amylase to other proteins.

Composition of Macroamylase

Most macroamylase complexes contain immunoglobulin complexed with normal amylase. IgA and IgG macroamylases have been observed. The composition of macroamylase is heterogeneous, and there is also evidence that polysaccharides and other proteins can be linked to amylase in some patients.

Immunoglobulin-Complexed Amylase

When macroamylasemic serum, or the amylase-free protein fractions from dissociated macroamylase, are mixed with normal serum amylase, a macroamylase complex is formed in most,† but not all[3,7,11,24] cases tested. The binding substance itself was first shown to be an immunoglobulin in 1968 when Levitt and Cooperband[7] reported a patient with an 11S macroamylase that was precipitated with anti-IgA antiserum. Since then, using immunoprecipitation and immunoelectrophoresis, 23 patients with IgA‡ and 12 patients with IgG[20,27,33,50,54,61] macroamylase have been reported. Two other macroamylases have been found to contain both IgA and IgG.[30,61] No IgM, IgE or IgD macroamylases have been found.

Immunoprecipitation studies of papain-digested macroamylase place the binding site for amylase in the Fab portion of the immunoglobulin and not the Fc portion.[19,50] Of 19 patients in whom the light chain has been identified, the light chain was exclusively kappa in nine and exclusively lambda in ten.[50,61] Immunoprecipitation may not detect globulins bound to amylase if they are cross-linked by disulfide bonds.[50]

Macroamylase immunoglobulins have variable affinities for the amylase of different species as well as for human pancreatic and parotid isoamylases. Levitt and co-workers[38] showed variable binding of four macroamylasemic sera to amylase from baboon and hog, but none to *Aspergillus* amylase. One patient who had been receiving hog pancreatic supplements had a higher molar affinity for hog amylase than human amylase, suggesting the possibility that he had become sensitized to the hog antigen and was producing IgA in the intestinal tract that cross-reacted with his own circulating amylase. Normal serum amylase is 44 percent to 73 percent S-type (salivary), and the remainder is P-type (pancreatic).[60] In a series of 30 macroamylasemic patients, the median percentage of S-type isoamylase in released amylase was 84 percent, with a range of 11 percent to 100 percent.[59] The binding substance in 26 of these cases had a higher affinity for S-type than P-type isoamylase. Kanno and Sudo[50] similarly studied five patients with macroamylasemia and reported higher S-type affinity in one and did not specify relative affinities in the others. Whether immunoglobulin binds at or away from the active site of amylase is not yet known.

Polysaccharide-Complexed Amylase

A synthetic macroamylase can be generated by mixing glycogen with human[12,13] or hog[68] amylase. Concanavalin A (Con-A) precipitates branched polysaccharides and has been shown to dissociate both uncharacterized macroamylase complexes[12,13] and documented immunoglobulin-amylase complexes.[50] It has precipitated one macroamylase complex[29] and not affected another.[13] In three patients soluble starch produced temporary dissolu-

*References 7, 10, 11, 13, 15-18, 20, 22, 26, 27, 38, 40, 50, 58-60.
†References 3, 8, 13, 16, 18, 20, 22, 27, 29, 38, 50, 60, 65, 66.
‡References 7, 18, 26-28, 31, 41, 46, 50, 54, 61, 67.

tion of macroamylase that was reversed after the starch was hydrolyzed.[29] Macroamylase was unaffected by other substances, including heparin,[12] dextran,[12,29] gelatin[29] and sucrose.[29] Thus, there are data suggesting that in some cases of macroamylasemia, amylase is bound at the active site by a polysaccharide or glycoprotein.

Additional evidence of macroamylase heterogeneity, not necessarily suggestive of polysaccharide binding, includes the failure to detect immunoglobulin linkage in 15 cases,[10,22,24,29,33,38,46,61] dissociation at neutral pH in two assays,[21,29] which is not characteristic of an antigen-antibody complex,[57] and incomplete or absent dissociation at acid pH.[13,15,29,38,60] A case of amylase complexed with α_1-antitrypsin has been reported,[33] but the physical properties of this macroamylase were not described.

Methods for Detecting Macroamylase

The definitive demonstration of an amylase complex with an abnormally large molecular weight requires fractionation of serum proteins by size and an assay of each fraction for amylase activity. Direct identification of macroamylase requires chromatography or ultracentrifugation. Gel chromatography can be adapted to a microcolumn or thin-layer filtration assay for rapid screening of sera. Indirect macroamylase assays such as the temperature-sensitive amylase activity assay, electrophoresis and isoelectric focusing, may be useful for mass screenings but may also be inaccurate in an individual patient. The pancreatic scan should not be used in macroamylasemia screening. Macroamylasemia can occur in the presence of normal serum amylase, but the combination of hyperamylasemia with an amylase: creatinine renal clearance ratio of less than 1 percent is indirect but highly suggestive evidence of macroamylasemia. Abdominal pain has been anecdotally linked with macroamylasemia, but statistical correlation is lacking; thus, it is concluded that macroamylasemia is asymptomatic.

Assays

Macroamylase can be identified within 10 to 60 minutes using a dextran gel microcolumn for protein separation followed by incubation of the large-sized fractions with amylose and iodine to assay for amylase hydrolysis activity.[1] Fridhandler and Berk[69] have distinguished three types of macroamylasemia with this method: (1) Type 1 is characterized by serum hyperamylasemia and detection of amylase activity within the first ten minutes of incubation; (2) type 2 displays serum hyperamylasemia, but more than ten minutes of incubation is required for detection of amylase activity, and (3) type 3 displays normal serum amylase, and more than ten minutes of incubation is required. Among these three groups, type 1 has the highest level of total serum amylase activity, the highest ratio of macroamylase to normal-sized amylase in the serum, the highest amylase activity in the macroamylase-containing fractions, and the most diminished levels of urine amylase compared with type 2, which is intermediate, and type 3, which is last in these areas.

Multiple sera can be screened simultaneously for macroamylase using thin-layer gel filtration with the amylase substrate and iodine applied directly to the gel.[21,70] Unhydrolyzed starch takes up stain, and zones with amylase activity remain white. Normal serum generates a single white stripe, and macroamylasemic serum generates two white stripes. The microcolumn and thin-layer chromatographic techniques for detecting macroamylase have replaced standard columns in most laboratories because they are quicker, easier and less expensive to run. Berggren and Levitt[40] reported a macroamylase, however, that was not detectable by using the microcolumn, but was shown by isoelectric focusing and standard column chromatography. This "unusual" macroamylase was only marginally larger than normal serum amylase and may not have been resolvable with ultracentrifugation. Macroamylase is generally reliably identified by ultracentrifugation,[16] although this technique is time consuming.

Electrophoresis has been advocated as a screening test for macroamylasemia.[27,48] A proper matrix needs to be devised that will impede the migration of macroamylase while allowing passage of all isoamylases of varying charges, to make this assay specific. Isoelectric focusing distinguished an abnormal amylase from one patient that was eventually found to be macroamylase,[40] however, macroamylase complexes usually do not focus clearly.[54] Both of these techniques can confuse differences in charge with differences in size; even so, electrophoresis is a potentially useful screening method.

An indirect temperature-sensitive method for determining the presence of macroamylase is based on the increased enzyme activity of serum amylase at 45°C compared with 25°C. In sera of normal subjects and patients with pancreatitis,

the increase is fourfold, but in patients with macroamylasemia it can be up to eightfold.[71,72] This screening method successfully identified a case of macroamylasemia in a random sampling of 100 hospital inpatients.[32,73] If macroamylase represents only a small proportion of a patient's total amylase activity, this indirect assay might fail to detect it. Brohee and co-workers[36] could not detect a chromatography-proved macroamylase with this method. In the presence of unexplained hyperamylasemia, a pancreatic scan has been advocated by Mark and McCord[74] to provide, if the results are normal, indirect evidence of macroamylasemia. Such a technique may be useful in excluding pancreatitis,[75] but it is much too indirect to be useful in implicating macroamylasemia because there are many other nonpancreatic causes of hyperamylasemia[76,77] and more specific methods for diagnosing macroamylasemia.

Serum Amylase

An elevated level of serum amylase is insensitive and certainly not specific for diagnosing macroamylasemia.[76,77] Although the vast majority of cases have been identified during evaluation of hyperamylasemia, Barrows and colleagues[78] and Helfat and co-workers[79] together screened for macroamylasemia in 1,052 patients with a variety of disorders, who were chosen at random with respect to their serum amylase level. Of these, 16 patients with macroamylasemia were identified; however, the serum amylase level was elevated in only 2 and was within normal limits in the other 14.[69]

Amylase Clearance:Creatinine Clearance Ratio

An abnormally low renal amylase clearance: creatinine clearance (C_{AM}:C_{CR}) ratio has been advocated as a diagnostic screening test for macro-

TABLE 1.—*Reported Mean Values for C_{AM}:C_{CR} in Normal Subjects Assayed by Chromogenic, Saccharogenic and Iodometric Methods*

Investigators and Reference No.	*Year*	*No. of Subjects (total)*	*Mean C_{AM}:C_{CR} (percent)*	*Product (total)*
Chromogenic				
Blainey & Northam[5]	1967	9	3.02	27.18
Warshaw & Fuller[84]	1975	46	3.10	142.60
Long & Grider[85]	1976	20	1.30	26.00
Morton et al[86]	1976	24	1.24	29.76
Donaldson et al[87]	1977	25	2.60	65.00
Levitt et al[82]	1977	10	0.80	8.00
Marten et al[88]	1977	87	3.02	262.74
Murray & MacKay[89]	1977	40	1.50	60.00
Pasternack & Klockars[90]	1978	13	2.10	27.30
Hegarty et al[91]	1978	26	2.31	60.06
		(300)		(708.64)
Mean			2.36	
Saccharogenic				
Levitt et al[92]	1969	36	2.30	82.80
Levine et al[93]	1975	12	2.15	25.80
Warshaw & Lesser[94]	1975	46	3.10	142.60
Warshaw & Lee[95]	1976	25	3.00	75.00
Johnson et al[96]	1976	20	2.40	48.00
Morton et al[86]	1976	24	1.46	35.04
Levitt et al[82]	1977	10	2.19	21.90
Farrar & Calkins[97]	1978	69	3.04	209.76
		(242)		(640.90)
Mean			2.65	
Iodometric				
Mulhausen et al[98]	1969	11	3.62	39.60
Levitt et al[82]	1977	10	1.52	15.20
Schiffer et al[83]	1977	5	3.84	19.20
		(26)		(74.00)
Mean			2.85	

C_{AM}:C_{CR} = amylase clearance:creatinine clearance (ratio)

amylasemia.[80,81] In normal control subjects this ratio ranges from 0.80 percent[82] to 3.80 percent.[83] In addition, it has been suggested that the amylase assay method used can produce significant differences in the C_{AM}:C_{CR} ratio.[82] There are three amylase assay methods in general use: chromogenic, saccharogenic and iodometric. Compiled data from surveys of normal control C_{AM}:C_{CR} ratios are summarized in Table 1. The mean normal C_{AM}:C_{CR} ratios for the three assays are as follows: chromogenic 2.36 percent, saccharogenic 2.65 percent and iodometric 2.85 percent. It is important to know the normal values for these assays because many reported macroamylasemic clearance ratios do not specify the assay method used or the associated control C_{AM}:C_{CR} values.

The C_{AM}:C_{CR} ratio is indeed significantly lower in macroamylasemic patients than in normal control subjects. In documented cases of macroamylasemia in which the clearance ratio was reported, it was less than 1 percent in 37 patients,* 1.01 percent in one patient[21] and greater than 1.01 percent in only four patients.[21,34,35,100] One of these four patients was a man with a posterior antral ulcer and back pain; the ratio in this patient ranged from 1.46 to 2.1 percent. The patient may have had superimposed mild pancreatitis, which can transiently raise the clearance ratio in a macroamylasemic person from less than 1 percent to 4.5 percent[40]; this patient was not retested later when he was asymptomatic. The other three of these four patients were screened together,[34,35,100] with 14 ratio determinations carried out. Only one was less than 1 percent; the other ratios were as high as 11 percent, with essentially the same spread as in a group of patients with acute pancreatitis. Control subjects were not tested, however, and no specific information was given on how the diagnoses were made.

An additional cautionary note about the sensitivity of the low clearance ratio is that it has only been applied to hyperamylasemic macroamylasemic patients. It may lack dependability in the absence of hyperamylasemia. Barrows and colleagues[78] and Helfat and co-workers[79] did not report clearance ratios in their screening studies that identified 14 normoamylasemic patients among the 16 cases of macroamylasemia found. At least 13 of the 16 patients had type 3 macroamylasemia,[69] which is usually associated with normal, rather than low, urinary levels of amylase. These patients might have had clearance ratios that overlapped with normal values. Nevertheless, except for four cases in which important data were lacking, the C_{AM}:C_{CR} ratio has served as a sensitive indirect screen in patients with hyperamylasemia.

All patients reported in the literature with simultaneous hyperamylasemia and a low clearance ratio who have been so screened have had macroamylasemia except for five[48,101] who had salivary type (S type) hyperamylasemia with no detectable macroamylase. Two of the five had salivary gland enlargement and the other three had no obvious cause for this condition.

The mechanism for the decreased renal clearance of macroamylase is thought to be the large molecular weight of these complexes. They generally elute with the 100,000 or higher MW globulins on chromatograms and are too large for glomerular filtration. Proposed mechanisms for the low clearance ratio with salivary type hyperamylasemia include relatively slow renal clearance of the salivary compared with the pancreatic isoenzyme,[85,90,91,102,103] decreased nonrenal catabolism of the S-type isoamylase,[101] and the hypothetic presence of a macroamylase that dissociated during its analysis.[48]

Brohee and Delcourt[104] have mathematically illustrated the extent to which addition of a nonfiltered macroamylase decreases the clearance ratio of amylase to creatinine. This is shown in the following equation, which I have modified slightly.

In the presence of macroamylasemia, the renal clearance ratio is

$$\frac{C_{TA}}{C_{CR}} = \frac{C_A + C_M}{C_{CR}} = \frac{C_A}{C_{CR}} = \frac{\frac{U_{TA}}{P_{TA}}}{\frac{U_{CR}}{P_{CR}}} = \frac{\frac{U_A}{P_A + P_M}}{\frac{U_{CR}}{P_{CR}}}$$

multiplying by 1

$$\frac{\frac{U_A}{P_A + P_M}}{\frac{U_{CR}}{P_{CR}}} \times \frac{\left(\frac{P_A + P_M}{P_A}\right)}{\left(\frac{P_A + P_M}{P_A}\right)} = \frac{\frac{U_A}{P_A}}{\frac{U_{CR}}{P_{CR}} \times \left(1 + \frac{P_M}{P_A}\right)} = \frac{C_A}{C_{CR}} \times \frac{1}{\left(1 + \frac{P_M}{P_A}\right)}.$$

$$\therefore \frac{C_{TA}}{C_{CR}} = \frac{C_A}{C_{CR}} \times \frac{1}{\left(1 + \frac{P_M}{P_A}\right)},$$

where U = urine concentration, P = plasma concentration, C = clearance, $_A$ = amylase, $_M$ = mac-

*References 2, 3, 7-9, 21, 28-31, 36, 38, 40, 41, 48, 92, 99.

roamylase, $_{TA}$ = total amylase = $_{M}$ + $_{A}$, $_{CR}$ = creatinine, $U_M = 0$, and $C_M = 0$.

From this equation it is apparent that as P_M increases $C_{AM}:C_{CR}$ decreases. The percentage of total serum amylase activity found within macroamylase has been measured at between 7 percent and 100 percent.[7,21,34,79,92] Using these values in the equation, one would expect to find urinary clearance ratios ranging from 93 percent down to 0 percent of normal. The actual observations of 0 percent to 40 percent of normal (assuming a normal ratio of 2.5 percent) probably relate to underestimation of the macroamylase component of total serum amylase activity. Levitt and coworkers[92] compared clearance ratios with the macroamylase component of total serum amylase activity in six patients and found a linear inverse relationship as the equation implies.

In the presence of hyperamylasemia, a $C_{AM}:C_{CR}$ ratio of less than 1 percent is thus a useful indirect screening method for macroamylasemia. Confirmation of the diagnosis, however, still requires a direct assay.

Clinical Characteristics

Abdominal pain was a presenting symptom in 37 patients with macroamylasemia,* although the cause of the pain often remained undiagnosed. This relationship may be biased because patients with abdominal pain are screened for hyperamylasemia more often than patients without this symptom; thus, more cases of hyperamylasemia are discovered among them. Macroamylasemia, as will be discussed in the next section, is more frequent in hyperamylasemic patients than in randomly selected subjects, so that any condition requiring amylase screening will seem to have an inordinately high frequency of simultaneous macroamylasemia. It has been suggested that macroamylase may be deposited in the pancreas and thus produce pain,[48] but there is no experimental evidence to support this hypothesis. The frequency of macroamylasemia among patients with and without abdominal pain has not been compared. No other symptom is associated with macroamylasemia, and until the aforementioned comparison is made, macroamylasemia cannot be considered to be symptomatic.

*References 14, 21-24, 32, 36, 39-41, 48, 67, 74, 99, 105.

Epidemiology

How common is macroamylasemia? This condition has been screened for in randomly selected patients irrespective of their serum amylase levels and in groups selected specifically for their normal or elevated serum amylase activities. Diagnostic methods have included microcolumn,[34,35,78,79,100] thin-layer chromatography,[21] temperature-activity ratio[72,73] and simultaneous presence of serum hyperamylasemia with $C_{AM}:C_{CR}$ ratio of less than 1 percent.[106] Although the last two methods are indirect and, thus, not absolutely conclusive in an individual patient, they are adequate for estimating frequency within a large population. The results of the surveys are summarized in Table 2. The frequency of macroamylasemia in randomly selected patients is 1.05 percent. Among people with normal serum amylase activities it is 0.98 percent, and among people with hyperamylasemia it is 2.56 percent.

Macroamylasemia occurs more often in males than in females. There are currently 194 cases of macroamylasemia reported in the worldwide literature.† The 195th case is described in this review. Of the cases, 99 were in males and 62 were in females; the sex was not reported in 33 cases. The relative frequencies according to sex

†References 2, 3, 7-10, 13, 14, 20-36, 39-41, 46-50, 55, 58, 61, 66, 67, 69, 72-74, 78, 79, 92, 99, 100, 105, 107.

TABLE 2.—*Frequency of Macroamylasemia*

Investigators and Reference No.	*Year*	*Method*	*Serum Amylase*		
			Random	*Normal*	*Increased*
Barrows et al[78]	1972	Microcolumn		9/868	1/23
Imrie & Henderson[73]	1972	Temperature ratio			0/39
Long & Kowlessar[21]	1972	Thin-layer column		0/55	3/51
Imrie et al[32]	1973	Temperature ratio	1/139		
Helfat et al[79]	1974	Microcolumn	7/612		1/10
Dreiling et al[106]	1974	Serum amylase, $C_{AM}:C_{CR}$	8/773		
Bindrich et al[34]	1976	Microcolumn			3/190
			16/1524	9/923	8/313
Total Frequency (percent)			1.05	0.98	2.56

$C_{AM}:C_{CR}$ = amylase clearance:creatinine clearance (ratio)

are 61 percent men and 39 percent women, a statistically significant difference ($P<0.005$). The preponderance in men is unexplained. Serum amylase is 16 percent higher in normal women than in normal men,[108,109] but perhaps frank hyperamylasemia occurs more frequently in men than in women, leading to more frequent macroamylase screening in men. Barrows and co-workers[78] and Helfat and colleagues[79] together screened a group of 1,052 patients with a variety of diseases. The group was equal in sex distribution and the patients had been selected irrespective of serum amylase levels. Of 16 patients with macroamylesemia, 15 were men.

Macroamylasemia is an acquired condition. The age at the time of discovery of the condition was reported along with the patient's sex in 98 of the 195 cases.* The average age at the time of diagnosis was 53.3 years in the 63 male patients reported and 49.6 years in the 35 female patients reported. The ages ranged from 8[67] to 77 years,[33,50] but most patients were in the fifth, sixth and seventh decades (Figure 1). Although neonates have a fully developed immunoglobulin production system,[110] macroamylasemia was not shown in umbilical cord blood from 200 newborn infants selected at random.[78]

Macroamylasemia occurs throughout the world. Racial distribution among American patients whose race was reported[9,10,20,21,39,74,99] included 22 whites, 8 blacks and 1 Native American. There are no reports of more than one case in the same family; 29 family members of four macroamylasemic patients were screened, but no additional cases were identified.[10,29,32]

The duration of macroamylasemia is variable, and its presence may reflect coexisting disease. Some patients had unexplained hyperamylasemia for over ten years before macroamylasemia was diagnosed[99]; presumably, this condition was present the entire time. Macroamylasemia has been documented to persist for at least 300 days[58] and 100 days[32] in two diabetic patients. Follow-up surveys of 19 patients known to have the condition, at least 17 of whom had chronic illnesses, showed persistence of macroamylasemia in all of four cases after a week or more,[79] in seven of nine after a month,[78] and in all of six after two to ten months.[21] Wilding and co-workers[2] described a woman in whom macroamylasemia was present for at least three months but disappeared a month before she died from chronic illness. Zeze and colleagues[33] described a woman whose macroamylasemia disappeared one month after choledocotomy. Hedger and Hardison[39] reported a case of transient macroamylasemia in a woman during a hospital stay for acute intermittent porphyria, but no time frame was reported. Thus, macroamylasemia can occur transiently during acute illness and chronically during prolonged illness, and may, therefore, represent a nonspecific response to disease. Additional cases followed for longer times will provide more information about the natural evolution of macroamylasemia.

*References 2, 3, 7, 8, 10, 14, 17, 20-33, 36, 39-41, 50, 58, 61, 67, 74, 79, 99, 105, 107.

Causes

Why does the body produce immunoglobulins or other substances that can bind serum amylase? Macroamylasemia has been reported with greater

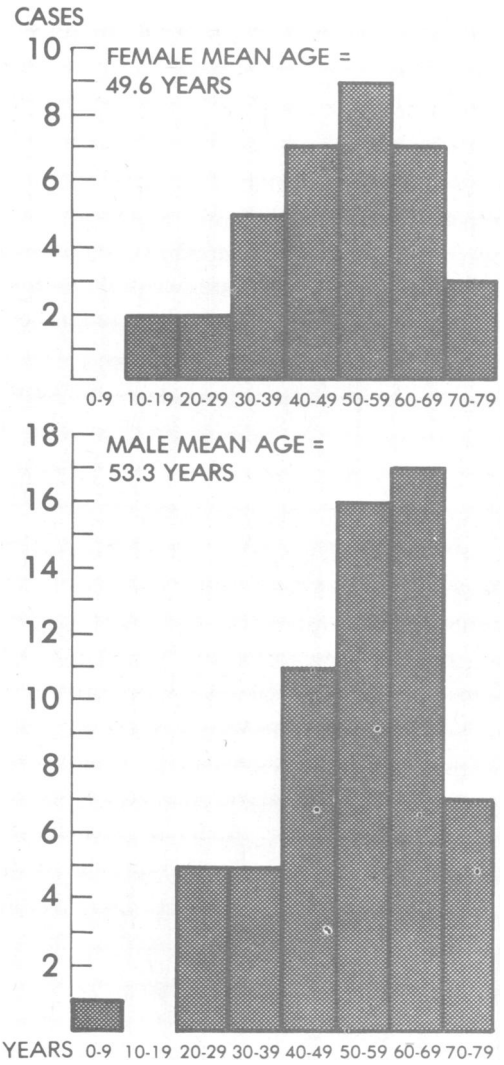

Figure 1.—Macroamylasemia—Age distribution by sex (including unpublished case).

frequency in patients in whom humoral immunity is disturbed, such as autoimmune diseases and cancer, or in patients in whom pancreatitis is a possible diagnosis, such as alcoholism, diabetes, cholelithiasis, malabsorption or pancreatitis itself. In the first setting, macroamylase appears to be a circulating complex of immunoglobulin and amylase, reflecting an aberrant state of immunoglobulin production. In the second setting there may be a spurious association because this group is generally screened for hyperamylasemia, whose presence also correlates with macroamylasemia. Macroamylasemia can also be iatrogenic.

Clinical Settings

The 108 patients with macroamylasemia whose clinical state has been described,* had isolated diseases of every organ system in the body; however, the following seven underlying disease states were found with some frequency.

Alcoholism. There were 19 cases of alcoholism coexisting with macroamylasemia.† In addition, seven patients with cirrhosis of the liver were reported although alcohol consumption was not mentioned.[32,33,35,50,61] Alcoholic patients are frequently screened for pancreatitis with serum amylase measurements and are occasionally found to have persistent unexplained hyperamylasemia that leads to macroamylase screening.

Pancreatitis. Eleven cases of acute or chronic pancreatitis with macroamylasemia were reported.[21,32,33,99] Close amylase surveillance in this group also leads to more frequent macroamylase screening, especially when hyperamylasemia persists out of proportion to the clinical condition.

Cancer. Eleven patients with macroamylasemia had cancer. The primary site was the lung in six cases,[35,50,79] the pancreas in two,[61] and the stomach,[50] esophagus,[50] and blood (leukemia)[48] in one each. Perhaps the tumors were associated with an abnormal antigen that cross-reacted with amylase.

Diabetes. Ten patients with diabetes,‡ including one with mumps-induced diabetic ketoacidosis,[58] had macroamylasemia. The factors that led to failure of the endocrine pancreas may also have subtly affected the exocrine pancreas leading to a mistaken recognition of amylase as a foreign substance stimulating production of amylase-binding immunoglobulin. In addition, diabetes can be a complication of pancreatitis, which is associated with macroamylasemia per se.

Cholelithiasis. Seven patients with cholelithiasis and macroamylasemia have been reported.[21,24,33,40,107] In addition, a 73-year-old Asian man with hyperamylasemia, cholelithiasis, a C_{AM}:C_{CR} ratio of 0.2 percent and macroamylasemia confirmed by microcolumn chomatography§ was seen at the University of California, San Francisco (Klonoff, unpublished observations). Most cholelithiasis workups include measurement of serum amylase, which can lead to discovery of inappropriate hyperamylasemia, and subsequent macroamylase screening.

Autoimmune Disorders. The first patient discovered to have macroamylasemia[2] died of a connective tissue disease characterized by fever, facial rash, phlebitis, leg ulcers and positive lupus erythematosus preparations. Six subsequent cases of autoimmune disorders associated with macroamylasemia have included systemic lupus erythematosus,[99] rheumatoid arthritis,[24] ankylosing spondylitis,[20] cryoglobulinemia,[41] monoclonal gammopathy[8] and heroin abuse.[79] The heroin addict, who also had bacterial endocarditis, had abnormal results of serology tests: positive latex fixation, and HBAg, and decreased serum complement. As mentioned earlier, a patient receiving hog pancreatic enzyme supplements for malabsorption had a macroamylase containing IgA with higher affinity for hog amylase than human amylase.[38] He may have become sensitized to hog amylase and produced antibodies to it that cross-reacted with human amylase. It is not surprising that macroamylasemia should be associated with disease states in which there are circulating immunoglobulins directed against a patient's own antigens.

Malabsorption. Four of the first ten macroamylasemic patients identified had a syndrome of malabsorption, atrophy of intestinal villi and macroamylasemia.[2,7,8,45] The presence of a small quantity of macroamylase, an IgA-amylase complex,[7] in the intestinal fluid of one of them led to speculation that the complex might be formed in the intestine from luminal IgA and amylase and that it might be cytotoxic and lead to atrophy of the villi. Only three additional macroamylasemic patients with

*References 2, 7, 8, 10, 14, 17, 20-26, 29, 32, 33, 35, 36, 39-41, 46, 48, 50, 55, 58, 61, 67, 74, 79, 99, 105, 107.

†References 10, 17, 21, 26, 32, 35, 36, 74, 79, 99.

‡References 17, 24, 32, 55, 58, 61, 79, 107.

§Kindly performed by J. Edward Berk, MD, Department of Medicine, University of California, Irvine.

malabsorption were reported subsequently,[22,40,99] and thus interest in this syndrome has waned.

The occurrence of macroamylasemia in these seven disease states may well reflect causes or effects of this condition. However, such relationships are speculative and are even less likely to be present for disease states with fewer reported cases. For example, after identifying macroamylasemia in a patient with a head injury, Imrie and co-workers[32] screened the next 20 neurosurgical admissions and failed to discover another case.

Iatrogenic Macroamylasemia

Infusion of hydroxyethyl starch (HES), but not dextran or gelatin, has produced serum macroamylasemia[37,42-44] with formation of an HES-amylase complex. No changes in serum levels of lipase, aspartate aminotransferase (formerly serum glutamic oxaloacetic transaminase), lactate dehydrogenase (LD), alkaline phosphatase, or gamma glutamyl transferase were noted. Serum amylase levels generally returned to the normal range within 72 hours in patients whose C_{CR} exceeded 10 ml per minute,[42-44] but in one patient[37] macroamylasemia persisted for four days.

Clinical Significance

The discovery of macromolecular amylase in 1964[2] established an additional cause of hyperamylasemia, but simultaneously raised several questions about the clinical significance of macroamylasemia. Who should be evaluated for macroamylasemia? What does it mean for a patient to have this condition? What should be done if macroamylasemia is diagnosed?

Any patient with asymptomatic hyperamylasemia and normal renal function (an important route of amylase elimination) should be evaluated for macroamylasemia. Detection of such cases most commonly occurs during automated screening of multiple chemistry values from a single blood specimen or during follow-up tests after clinical resolution of pancreatic or parotid disease. Because most hospital laboratories do not assay for macroamylase at present, the simplest confirmatory screening method (as mentioned earlier) is simultaneous determination of serum amylase and C_{AM}:C_{CR} ratio. Identification of macroamylase can short circuit an otherwise prolonged and expensive workup for hyperamylasemia.

Macroamylasemia occurs with a variety of diseases but with apparently increased frequency in states of altered immunity such as autoimmune diseases and cancer. The occurrence of macroamylasemia in apparently healthy persons as well suggests that it may be an early sign of disease, either as a marker for particular groups of diseases or as a nonspecific disease-induced dysproteinemia with amylase-binding capability. This possibility is reinforced by the correlation in some patients between the appearance or disappearance of macroamylasemia and the extent of coexisting disease. Whether the presence of macroamylasemia predicts any of these conditions, or its disappearance predicts recovery[33,39] or death[2] is not known. Long-term follow-up of apparently healthy macroamylasemic persons is needed to see whether a particular disease develops or not. A macroamylase assay might someday serve as a screening test for diseases of altered immunity.

Macroamylasemia is a benign condition that does not require treatment. When macroamylasemia accompanies systemic disease, it is a by-product of the disease state and all treatment should be directed at the underlying problem. Although macroamylase has been found in the duodenal aspirate of one patient with atrophy of intestinal villi and malabsorption,[7] causal relationship was found. It has been speculated but not documented that macroamylasemia causes abdominal pain.[48] If macroamylase were independently cytotoxic, one might expect it to bind complement; however, its complement-binding potential has not yet been tested. A patient with the triad of unexplained abdominal pain, hyperamylasemia and macroamylasemia is often subjected to exploratory laparotomy for examination of the pancreas. This is nonproductive and should be avoided. Fifteen patients with this triad were subjected to laparotomy[9,21,32,74,99] and no abnormality of the pancreas was found in 13, although no biopsies were obtained. The 2 patients with a visibly abnormal pancreas were the only patients among the 15 with clinical pancreatitis, acute in one case[21] and chronic in the other.[99] Thus, laparotomy showed no gross pancreatic pathology in all hyperamylasemic patients who had macroamylasemia without clinical pancreatitis. If macroamylasemia is found to be a harbinger of eventual development of systemic disease, then the approach to the macroamylasemic patient will have to include appropriate follow-up and screening for the associated disease. Without confirmation

of such an association, macroamylasemia should be regarded as a benign condition that requires no special management or treatment.

Other Immunoglobulin-Complexed Enzyme Disorders

To the extent that macroamylase represents a phenomenon in which immunoglobulin is complexed with a circulating enzyme (amylase), it is not unique. Lactate dehydrogenase, alkaline phosphatase, creatine kinase (formerly creatine phosphokinase), alanine aminotransferase (formerly serum glutamic pyruvic transferase) and glucose-6-phosphate dehydrogenase have also been found complexed with immunoglobulin. A designation of this phenomenon as an immunoglobulin-complexed enzyme (ICE) disorder is suggested. Each of the ICE disorders represents a possible cause for an unexplained elevated level of a serum enzyme. Immunoglobulin-complexed nonenzymatic proteins have also been reported. The ICE disorders represent an incompletely understood dysproteinemic response of the human immune system to a variety of disease states.

MacroLDemia

An isolated elevated serum LD level in a healthy person is often dismissed as "nonspecific." The patient is less likely to undergo an extensive workup if he has elevated LD activity than if he has elevated amylase activity because there are so many more causes for the former, and each diagnostic test is likely to have a low yield. The presence of macromolecular LD is yet another cause of elevated serum LD levels.

After amylase, LD is the next most common enzyme found in an immunoglobulin-complexed state. The presence of a circulating macromolecular LD is analogous to that of macromolecular amylase, which is designated as macroamylasemia. Similarly, macromolecular LD can be designated as "macroLD" and the presence of circulating macroLD as "macroLDemia." This condition does not appear to cause or be associated with any symptoms or particular diseases. Its clinical significance, like that of all the ICE disorders, appears to lie in its tendency to be an often overlooked cause of an elevated serum enzyme level, in this case LD. MacroLDemia, however, is associated with the presence of antinuclear antibodies and, therefore, indirectly with autoimmune disease.

The physical properties of macroLD are heterogeneous. Lactate dehydrogenase is a tetramer of two types of subunits, H and M, and can be separated electrophoretically into five isoenzymes (H_4, H_3M, H_2M_2, HM_3 and M_4). Each subunit has an MW of 34,000 and each isoenzyme has an MW of 134,000.[111] Forty-five patients with the abnormal macromolecular form of LD have been identified.[112-126] MacroLD was discovered during analysis of sera from patients with persistently elevated serum LD activity. The macroLD differs from all normal LD isoenzymes in its chromatographic, electrophoretic and kinetic properties. Whereas normal LD elutes from Sephadex G-200 gel between albumin and gamma globulins, in 42 patients LD activity eluted early, between the macroglobulins and gamma globulins, in addition to* or instead of[114,115,119,122,126] normal LD. Two macroLD complexes dissociated to normal size during gel filtration[119] and one was not tested.[120] The MW of the macroLD has been estimated at 200,000 to 400,000.[115,125,126] Electrophoresis of the macroLD showed pronounced heterogeneity among the patients. In each case there was at least one extra abnormal band, as well as a deficiency of one or more of the normal LD isoenzymes. Abnormal large MW fractions on the chromatogram contained the abnormal LD fractions seen with electrophoresis. The physical properties of the macroLD complex are heterogeneous but differ from normal LD and usually each other in their heat stability† and their responses to nicotinamide adenine dinucleotide,[116,118,119,123] mercaptoethanol,[118,119] $(NH_4)_2SO_4$,[120] pyruvate[125] and urea.[125]

MacroLD contains immunoglobulin complexed to LD in most cases studied (Table 3). Among the 45 cases identified, immunoelectrophoresis detected 24 cases of IgA linked to LD,[114,116-118,120-122] 16 cases of IgG linked to LD[115,119,123,125] (14 of these were of the subclass IgG_3 and two were not subclassified), and one case of both IgG_1 and IgA linked to LD.[123] Both kappa and lambda light chains have been noted either alone or together in the same patient.[119-123,127] Hemagglutination inhibition may be a more sensitive assay than immunoelectrophoresis or light chain analysis.[127]

*References 112, 113, 116-118, 121-125.
†References 112, 116, 118, 119, 123-125.

Although theoretically immunoglobulins directed against an LD isoenzyme that contains both subunits might precipitate all five isoenzymes containing one or both of these subunits, antibodies directed exclusively against one hybrid isoenzyme have been observed.[122,128] Further studies are needed to locate the immunoglobulin's site of binding to LD and to explain how a single hybrid isoenzyme can be bound selectively.

Elevated immunoglobulin levels may contribute to formation of macroLD but are not essential. Quantitative immunoglobulins were measured in 11 patients with IgA-LD complexes and IgA was elevated in five of them.[114,118,120,121] They were also measured in six patients with IgG-LD complexes and IgG was elevated in four of them.[115,119,123,125]

MacroLD is an acquired disorder. The abnormal properties of macroLD are due to interaction with some substance in the serum, rather than to a genetically abnormal structure. This conclusion is based on four observations: (1) The LD from erythrocytes, leukocytes, and lymph node extract is normal in patients with macroLD in the serum,[113,116-121,123] although salivary IgA-LD complexes were found in one of two patients screened.[118,121] (2) Serum specimens from these patients mixed with normal human serum[114-120,122-126] or horse, cow, hog or rabbit serum[122] reproduces the electrophoretically abnormal LD rather than producing two LD families. (3) The serum LD abnormality can be transient.[118-120,123,124,126] (4) Abnormal circulating LD has not been detected among family members of affected patients.*

Establishing the diagnosis of macroLDemia requires chromatographic or immunologic evidence of an abnormal LD complex. Ultracentrifugation has not been used to study macroLD but would be a definitive diagnostic method. An elevated serum LD level is not specific for diagnosing macroLDemia, and is also insensitive because 5 of the 45 patients with macroLDemia had normal levels of LD.

The frequency of an electrophoretically abnormal LD has been estimated as one in 2,500[113] and five in several thousand.[129] The frequency of an immunoglobulin-complexed LD has been estimated at less than one in 10,000.[118] Biewenga and Feltkamp[123] screened sera from 100 patients with rheumatoid arthritis and identified a single case of macroLDemia, but found no cases among 19 patients with systemic lupus erythematosus. Twenty-two cases each have occurred in men and women; the sex of another patient was not disclosed. The mean age at the time of diagnosis was 58.7 years, and the age distribution was bimodal, with a small peak in the third decade and a large peak in the seventh decade (Figure 2). No particular disease state is associated with macroLDemia, but in 13 of the 45 patients antinuclear antibody studies were positive, suggesting an association with systemic autoimmune disease.

Circulating LD can thus be found in a macro-

*References 112, 113, 116, 118, 121, 126.

TABLE 3.—*Cases of MacroLDemia*

Investigators and Reference No.	*Year*	*Previously Unreported Patients*	*Molecular Weight*			*Immunoglobulin-LD Complexes*					*Elevated Serum LD*	*Positive ANA*
			Elevated	*Normal*	*Untested*	*IgA*	*IgG*	*IgA+ IgG*	*Absent*	*Untested*		
Lundh[112]	1967	1	1	..	..	..	..	..	1	..	1	..
Voigt[113]	1967	1	1	..	..	..	..	..	..	1	1	..
Ganrot[114]	1967	1	1	..	..	1	..	..	..	..	1	1
Kindmark[115]	1967	1	1	..	..	..	1	..	..	..	1	1
Biewenga & Thijs[116]	1970	1	1	..	..	1	..	..	..	..	1	..
Nagamine[117]	1972	2	2	..	..	2	..	..	..	..	2	..
Biewenga[118]	1972	7	7	..	..	7	..	..	..	..	7	..
Biewenga[119]	1973	3	1	2	..	..	3	..	..	..	3	2
Markel & Janich[120]	1974	1	..	..	1	1	..	..	..	..	1	..
Thomas et al[121]	1974	1	1	..	..	1	..	..	..	..	1	..
Biewenga & Feltkamp[122]	1975	11	11	..	..	11	..	..	..	..	9	2
Biewenga & Feltkamp[123]	1975	12	12	..	..	..	11	1	..	..	10	7
Tanaka et al[124]	1976	1	1	..	..	..	..	..	1	..	1	..
Hayashi et al[125]	1976	1	1	..	..	..	1	..	..	..	1	..
Meaney et al[126]	1976	1	1	..	..	..	..	..	1	..	..	..
TOTALS		45	42	2	1	24	16	1	3	1	40	13

ANA = antinuclear antibody, LD = lactate dehydrogenase

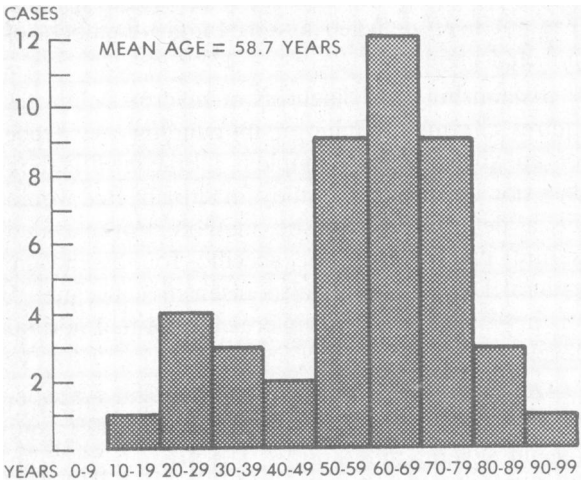

Figure 2.—MacroLDemia—Age distribution.

molecular form that is usually complexed with an immunoglobulin and has heterogeneous physical properties; this form can be designated as macroLD. The presence of macroLD or macroLDemia appears to have clinical significance similar to that of macroamylasemia.

Other Enzymes

Serum enzymes other than amylase and LD have been found linked to immunoglobulins. Four patients with circulating IgG-alkaline phosphatase complexes have been reported.[130,131] The IgG light chains were lambda in all four. The bound alkaline phosphatase was hepatic in two cases, osseous in one, and both hepatic and osseous in one. Human intestinal and placental as well as other mammalian alkaline phosphate isoenzymes were not bound by the patients' sera. Three of the patients had an elevated serum level of alkaline phosphatase and two had osteoporosis. The frequency of this disorder is estimated to be 0.1 percent of patients with an elevated serum level of alkaline phosphatase.[131] Urdal and Landaas[132] reported six patients with macromolecular complexes of the creatine kinase (CK) BB isoenzyme, or macroCK. In four cases the complex contained IgG, and in two no immunoglobulin could be demonstrated. The patients had no clinical disorder in common. An incidence of five cases of circulating macroCK among 310 randomly screened sera specimens was reported, and three of the five were due to IgG-CK complexes. It is noteworthy that immunoglobulin-bound CK-BB can be measured as MB in an ion exchange column chromatography assay, leading to a possible misdiagnosis of myocardial infarction. Kajita and co-workers[133] screened 500 patients with chronic liver disease and found a circulating IgG-alanine aminotransferase complex in 16 of them. Fifteen of the 16 had an elevated serum level of alanine aminotransferase. In a report by Eng[134] there were five patients with electrophoretically abnormal glucose-6-phosphate dehydrogenase that was bound to an uncharacterized substance in the serum. One of the patients had cancer of the penis, one had hemolytic anemia and one had fever of obscure origin; two were screened from among 131 apparently healthy volunteers.

Nonenzyme Proteins

The protease inhibitor α_1-antitrypsin has been reported to be complexed with IgA in patients with IgA myeloma, although the serum level of this protein was not reported in any of the cases and therefore it is not known if the level was elevated.[135-137] Complexes of IgA and α_1-antitrypsin may constitute 1 percent of plasma IgA.[136] Immunoglobulins complexed with a variety of other nonenzymatic proteins have also been reported, including albumin[64,138-142] and haptoglobin.[138] Rheumatoid factor[143] and mixed cryoglobulins[144] can be considered as immunoglobulin-complexed immunoglobulins. Immunoglobulins have spontaneously developed against multiple coagulation factors[145] and peptide hormones, such as insulin,[146-148] glucagon[149] and human chorionic gonadotropin-luteinizing hormone,[150] in nonsensitized patients.

Conclusions

The ICE disorders represent a small but growing group of phenomena in which an immunoglobulin is complexed with a circulating serum enzyme or nonenzyme protein to create a macromolecular complex. Macroamylasemia is the most extensively studied member of the group. If the factors leading to synthesis of ICE immunoglobulins could be modified to produce immunoglobulins specifically directed against antigens of tumors or infectious agents instead, then the lessons learned from ICE disorders could help treat numerous diseases.

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