Immunoassay of P-Component in Amyloidotic Sera.* (32292)

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To date, all human amyloidotic spleens studied by us have yielded a protein which is not detectable in normal or non-amyloidotic tissue. This protein has been identified by immunoelectrophoresis in the blood of normal individuals and for this reason has been called the P (plasma)—component of amyloid(1,2). We have recently measured the plasma level of P-component in patients with amyloidosis in order to determine whether its presence has any diagnostic significance and whether any data relevant to the genesis of amyloid might be obtained. Sera from patients with rheumatoid arthritis were studied as "pathologic" controls and also because this condition is not infrequently complicated by amyloidosis.

Materials and Methods. A total of 35 pathologic sera were tested; 21 were derived from patients with biopsy proven amyloidosis, the remaining 14 from patients with classical rheumatoid arthritis. As controls, sera from 25 young and presumably healthy blood donors and cord blood from 9 new-born infants were tested. Serum was stored at —30°C and thawed just before use. An antiserum specific for P-component was prepared in rabbits from extracts of amyloid-laden tissues as previously described(3).

Immunoassays of P-component in serum were performed in Oudin tubes according to the method of Huntly(4). For these determinations a specially prepared glycine extract, pH 9.5, from an amyloid laden spleen was arbitrarily considered to have 1,000 units of P-component per 100 mg starting material and used as a reference sample for subsequent tests on serum.

Results. In 23 blood donors the mean serum level of P-component, 103 units/100 ml (S.D. ± 22) was remarkably similar to that of the 14 patients with rheumatoid arthritis, 102 units/100 ml (S.D. ± 30). Serum levels of P-component in patients with amyloidosis, taken as a whole, did not differ significantly from those of the blood donors and rheumatoid arthritis patients but the standard deviation and range of serum levels in amyloidosis were greater than in normal controls and rheumatoid arthritis (Table I). Cord serum was remarkable in that 5 of 9 bloods tested showed no detectable P-component and in the remaining 4 the levels were equal to or less than 40 units/100 ml, a level which is under the 95 percentile for normal and rheumatoid serum (Fig. 1).

Serum levels of P-component were also considered separately with respect to major clinical classifications of amyloidosis. Unfortunately the numbers in each classification tested were small, but it was noteworthy that the mean for 9 patients with secondary amyloidosis was similar to that of controls whereas the mean for patients with primary amyloidosis and that associated with multiple myeloma were approximately 20 units/100 ml lower (Table II). The range of serum levels of P-component was extremely variable, however, even in secondary amyloidosis. Several cases were found in which the values exceeded the 95 percentile for normals, at both the upper and lower levels (Fig. 1).

Discussion. The pathogenesis of amyloid is not known. Prevalent theories have included the concept either of local formation of amyloid or of deposition of a plasma component in tissue. It has also been suggested that locally precipitated amyloid can be partially solubilized(5,6). Several studies have shown that an unusual plasma protein can be identified in extracts of amyloidotic tissues but not in similarly prepared extracts of normal or non-amyloidotic tissue(1,7,8). Little is known concerning the levels of this substance in normal or pathologic serum, although one

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TABLE I. Mean Serum Levels of P-component in Patients with Amyloidosis and Controls.

	No. of cases	Units/100 ml*
Amyloidosis	21	87 ± 42
Rheumatoid arthritis	14	102 ± 30
Healthy blood donors	23	103 ± 22
Cord blood	9	11†

* The difference between the means for patients with amyloidosis and healthy blood donors was not significant ($.05 < P < .10$).

† In 5 cord bloods there was no detectable P-component.

author claims to have identified an alpha globulin obtained from amyloid deposits which is present in normal serum but increased in serum of patients with multiple myeloma, macroglobulinemia and amyloidosis (9). In contrast, preliminary studies performed by us originally showed only trace amounts of P-component in normal and amyloidotic serum but no attempt was made to measure serum levels of this substance by a sensitive technique. In the present investigation we have employed an immunoassay which allows for more accurate determination of P-component in serum and we have extended our observations to include a far larger number of cases of amyloidosis and controls than was previously tested.

The results showed somewhat unexpectedly that serum levels of P-component in amyloidosis did not differ significantly from those obtained in patients with rheumatoid arthritis and in healthy blood donors. Patients with primary amyloid, with senile amyloid and with amyloid associated with multiple myeloma tended to have lower values than patients with secondary amyloid. The variation of serum levels of P-component within the entire group of patients with amyloidosis was greater than that of the controls. For unexplained reasons, several patients with amyloidosis had only minimal serum levels of P-component and in this regard resembled new-born infants in whom 5 of 9 bloods tested had no detectable P-component and the remainder had exceedingly small amounts.

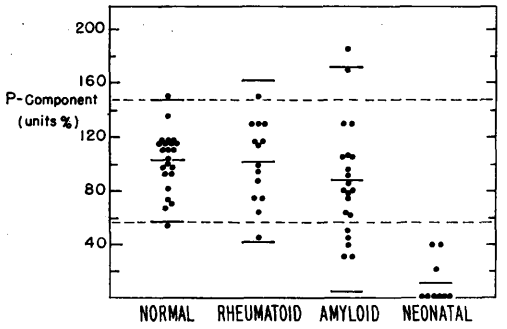

FIG. 1. Range of serum levels of P-component in normal, rheumatoid, amyloid and neonatal subjects. Two standard deviations for normal subjects are indicated by the broken lines.

From a practical viewpoint the results of the study were disappointing in that they seemed to rule out the determination of P-component levels in serum as a useful diagnostic test for amyloidosis. For theoretical reasons, the results were more interesting. If P-component is related to amyloid deposition, as studies carried out by us so far suggest, then its presence in normal serum indicates that at least one constituent of amyloid may possibly be found in normal connective tissue by appropriate techniques. The low values obtained in new-born infants further support the concept that amyloid development may be a function of aging in man as well as in animals. Finally, while the similar levels of P-component in various types of amyloidosis point to a common mechanism in the laying down of amyloid deposits in these conditions, the minor differences and variation found will be worthy of further study. More extensive investigation, including the purification of P-component, and determination of its molecular configuration will be necessary before we can ascertain whether this unusual alpha-globulin is an integral part of the amyloid fibril or whether it is one of several components uniquely found in amyloid.

TABLE II. Mean Serum Levels of P-component in Patients with Different Types of Amyloidosis.

	No. of cases	Units/100 ml
Primary	5	76
Myeloma associated	4	81
Secondary	9	104
Senile cardiac	1	45
Lichen	2	79

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Influence of 5-Fluorouracil on Thyroid Function.* (32293)

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5-Fluorouracil (5-FU) inhibits fetal growth, growth, liver regeneration, testosterone-induced growth of seminal vesicles, and somatotropin-induced growth of the epiphyseal cartilage(1); however, it increases the adrenal size presumably by stimulating secretion of ACTH(2). Acute experiments indicate that other antimetabolites such as Actinomycin D(3) and Puromycin(4), which interfere with protein synthesis, do not alter thyroidal iodine metabolism. Experiments were designed to determine if treatment with 5-FU, in doses that inhibit the growth of certain transplantable tumors when given for 5 or 10 days, would alter the effects of TSH on thyroid weight or iodine metabolism.

Material and methods. Four groups of male rats (Barkbridge Farms, N.J.) weighing about 150 grams were used. Each group consisted of 5-FU-treated and control animals; the latter differed from the former only in that they did not receive the antimetabolite. Group 1 had free access to laboratory chow and tap water and were injected with 25 mg/kg body weight of 5-FU for 5 days. Group 2 consumed an iodine-deficient diet and distilled water for 21 days and were injected with 5-FU, 25 mg/kg body weight, for the last 5 days of the experimental period. Group 3 were injected with 12.5 mg/kg body weight of 5-FU daily for a 10 day period during which they were given the iodine-deficient diet and distilled water. Group 4 received laboratory chow and tap water and were given 25 mg/kg body weight of 5-FU for five days and 8 U of TSH daily for the last four days, the last dose of each being given 24 hrs before death of the animals.

24 hours prior to sacrifice each animal was injected with one microcurie of I-131. At the time of sacrifice the thyroid glands were dissected and weighed, and the radioactivity was measured in a well counter. The circulating radioactive compounds of the animals in Group 2 (iodine-deficient for 21 days) were identified by radiochromatography using the butanol-ethanol-ammonia system.

Results and discussion. The results are listed in Table I. 5-FU in a dose of 25 mg/kg body weight for 5 days did not significantly alter the thyroid weight or the I-131 accumulation of rats fed either laboratory chow or an iodine-deficient diet for 21 days (Groups 1 and 2); however the I-131 uptake of the 5-FU treated iodine-deficient animals tended to be higher than that of their controls. Results of radiochromatographic analysis of the plasma of the 21 day iodine-deficient animals were the same in the 5-FU treated animals as in the controls. The ratio of radioactivity corresponding to thyroxine to that of inorganic iodide was 4:1, lesser amounts ran

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