THE PLASMA DISAPPEARANCE TIME AND CATABOLIC HALF-LIFE OF I^{131}-LABELED NORMAL HUMAN GAMMA GLOBULIN IN AMYLOIDOSIS AND IN RHEUMATOID ARTHRITIS *

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In 1906, Lewis (1) suggested the possibility that an abnormality in globulin metabolism might play a role in the pathogenesis of amyloidosis because he observed that hyperglobulinemia regularly occurred in horses that developed amyloid as a result of tetanus toxin administration. A similar association between prolonged hyperglobulinemia and the ultimate development of amyloidosis has been noted in a variety of laboratory animals (2–4) and in human beings having either the primary form of the disease (5) or that secondary to rheumatoid arthritis or other chronic inflammatory conditions (6). Whether this association is merely coincidental or of pathogenic significance is not yet known.

While no one globulin fraction has been found to be elevated in all instances and types of amyloidosis, γ-globulin is frequently increased early in the course of the disease and its concentration may decrease during the period of rapid accumulation of amyloid (7). Vasquez and Dixon (8) have found that amyloid, both in humans and in animals with experimental disease, contains γ-globulin which may be revealed by the Coons fluorescein-labeled antiglobulin technique. On the other hand, studies by Calkins, Cohen and Gitlin (9), using an immunochemical technique, have failed to demonstrate γ-globulin in amyloid. Because of these conflicting observations, it was felt that a study of the fate of intravenously injected, labeled γ-globulin in subjects with amyloidosis might throw further light on the relationship between circulating γ-globulin and amyloid. A group of patients with active rheumatoid arthritis was also studied, with the assumption that an illness known to predispose to the development of amyloidosis might reveal a similar alteration in γ-globulin metabolism.

MATERIALS AND METHODS

In the present study, the rate of disappearance from the circulation of intravenously administered I^{131}-labeled γ-globulin, its catabolic half-life, and distribution space were determined. Subjects studied included 4 patients with rheumatoid arthritis, 3 with amyloidosis and 3 normal controls. One of the patients with amyloidosis was first studied as an arthritic. After the first study he developed manifestations of amyloidosis and was reinvestigated 1 year later. Brief case summaries of these patients are appended. The laboratory data are given in Table I.

Labeling of the γ-globulin with I^{131} was carried out by the method of Gitlin, Janeway and Farr (10), a modification of that of Pressman and Eisen (11). The protein employed was poliomyelitis immune globulin prepared by Cutter Laboratories. Four lots of labeled protein were prepared in the course of the study. Although the specific activity and the protein concentration of the material varied from lot to lot (see Table II), the amount of total iodine relative to protein was calculated in such a way that a maximal labeling of 3 atoms of iodine per molecule of protein was anticipated. After dialysis against saline, the protein solution contained less than 1 per cent nonprotein-bound iodine. No iodine was released from the protein after storage of the solution at 4° C for 30 days. Paper electrophoresis of the protein after storage and radiography using photographic film, revealed a homogeneous material containing radioactivity only in the γ-globulin band.

Each subject studied received 24 to 60 μc of radioactive iodine contained in about 20 mg of γ-globulin. After an intravenous infusion of the protein, serum samples were obtained daily for the first 2 weeks and on alternate days thereafter for a total period of 1 month. In addition, during the first 2 days of the experiment, serum was obtained at more frequent intervals, as may be seen in Figure 1. The total amount of blood withdrawn from each

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TABLE I

Pertinent laboratory data for subjects used in this study

Subjects	Age	Duration of disease	Involvement	Associated disease	Total protein	Albumin	γ-globulin*	NPN
	yrs	*yrs*			*g %*	*g %*	*g %*	*mg %*
1. Amyloidosis								
G.P.	55	2	Skin, heart	None	6.8	3.5	0.5	20
N.S.	38	1	Kidney, liver	Rheum. arthritis	8.8	2.9	3.1	23
M.F.	50	1	Kidney	None	3.1	0.6	0.5	23
2. Rheum. arthritis								
B.S.	60	34	Hands, feet spine	None	7.8	3.6	1.3	28
J.P.	61	9	All joints	None	9.8	4.9	2.1	29
L.S.	62	8	Hands, feet spine	Diabetes mellitus	8.2	2.6	3.0	33
3. Normal								
F.T.	63				6.6	4.5	2.1	29
J.G.	48			Old poliomyelitis	6.4	4.4	1.8	
F.W.	41				7.0	4.8	2.0	27

* Calculated from paper electrophoresis.

subject over 1 month was about 150 ml or 3 per cent of the total blood volume. Serial 24-hour urine collections were made for as long as radioactivity could be detected. From the pooled urine specimen for each 24-hour period, an aliquot was taken for counting. When proteinuria was present, all urine samples were counted before and after precipitation of the protein with trichloroacetic acid. A correction for the excretion of unmetabolized labeled protein was made in subsequent calculations (see Appendix). All subjects received Lugol's solution, 30 drops per day orally, in order to minimize thyroidal uptake of the radioiodide. Counting was performed on 1- or 2-ml samples in a β-sensitive, plastic well scintillation counter (12).

Data and calculations

The calculations employed in this study are conventional (13) and will be briefly illustrated with reference to the data obtained from one control subject, F.W. (Figure 1).

A. The half-time of disappearance of isotopically labeled γ-globulin from serum. The half-time of disappearance is represented by the slope of the final, most nearly linear, phase of the curve obtained when serum activity per unit volume is plotted logarithmically on the ordinate against time on the abscissa. In order to compare the curves for different subjects, the daily serum activity for each subject was plotted as a fraction of the initial (10-minute) value (Figure 2).

B. Catabolic half-time. This value is obtained by plotting total nonprotein-bound urine radioactivity per 24-hour period, as for serum activity. The slope of this curve represents the rate of breakdown of the injected protein, since the iodine label is promptly excreted when catabolism takes place (13).

C. Activity retained. If urine activity is subtracted daily from the total radioactivity administered, a curve representing the activity still present in the body for each day is obtained. The slope of this curve also yields the catabolic half-time. As may be seen in Figure 1, it parallels the slope of the curve obtained directly from the urine excretion.

D. Distribution space. If the amount of activity retained in the body on any day is divided by the serum activity per unit volume for that day, the total space of distribution of the labeled protein may be calculated. This calculation is subject to several theoretical and practical difficulties, as will be shown.

RESULTS

Figure 2 presents the serum γ-globulin disappearance curves for each of the subjects in this study. All the curves showed an initial rapid decline before becoming linear at about Day 14. After this time it may be seen that all but three are parallel. These clearly show a more rapid serum disappearance (N.S.2, L.S., M.F.).

Table III presents the data for the entire group in numerical form. The patients are grouped according to the different lots of iodinated γ-globu-

TABLE II

Protein concentration and specific activity of lots of iodinated protein; dose administered to each subject

Lot no.	Date	Subject	Protein conc.	Specific activity	Amount inj.	Activity inj.
			mg/ml	*μc/mg*	*ml*	*μc*
1	7/57	G.P.	8	1.5	4.7	56.4
		N.S.			5.0	60.0
2	1/58	B.S.	20	1.7	0.78	26.7
		J.P.				
3	5/58	L.S.	20	3.8	0.74	57.0
		F.T.				
4	8/58	N.S.	20	1.2	0.99	23.8
		M.F.			1.0	24.1
		J.G.			0.56	13.5
		F.W.			1.0	24.1

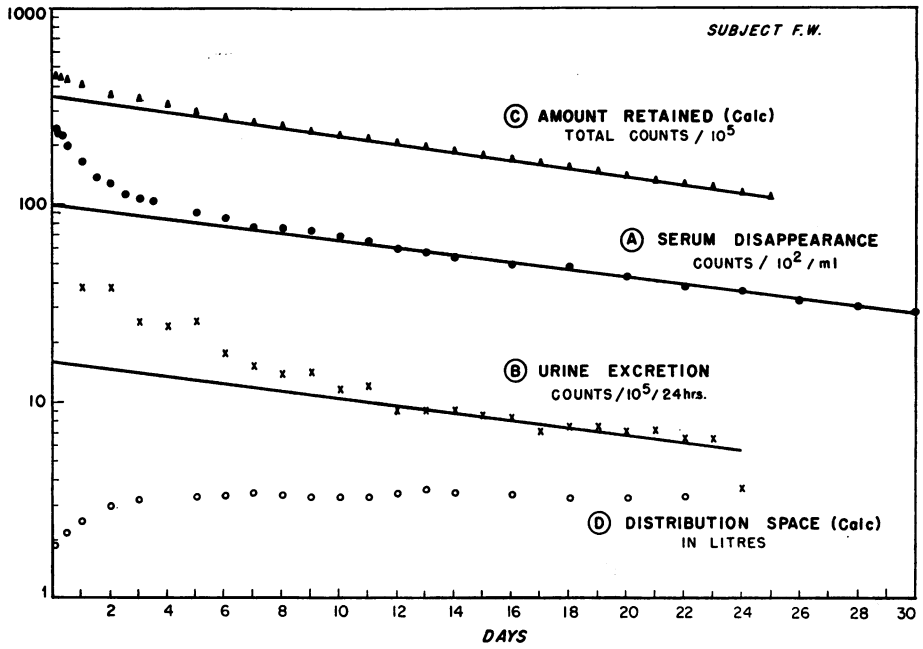

FIG. 1. PARAMETERS STUDIED IN 1 NORMAL CONTROL SUBJECT (F.W.). **A.** Daily serum activity measures as counts (in hundreds) per milliliter. **B.** Activity excreted in the urine for each 24 hour period (counts/10^5). **C.** Total activity retained in the body calculated for each day of the study (counts/10^5). **D.** Calculated space of distribution of the labeled protein (in liters).

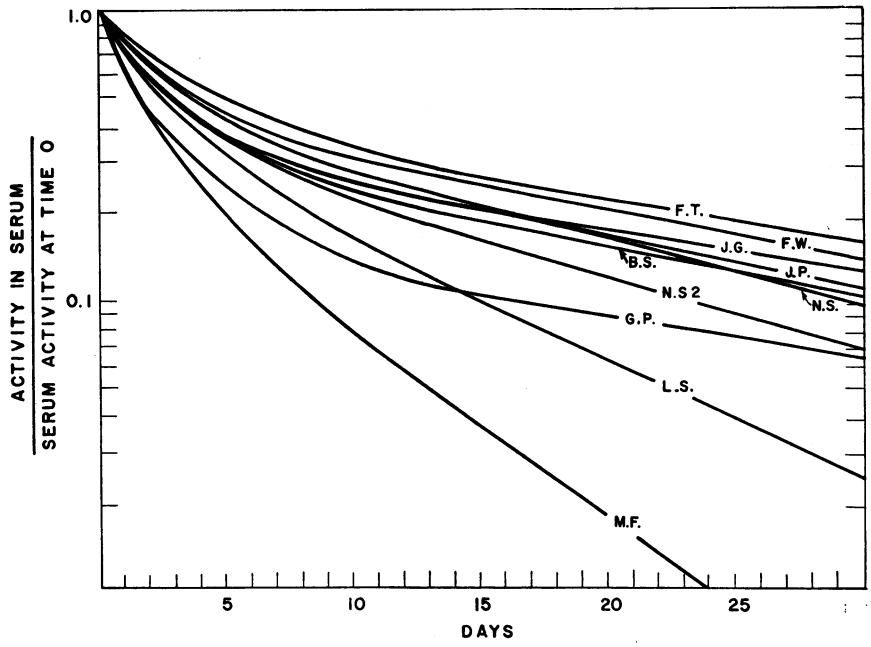

FIG. 2. CURVES OF DISAPPEARANCE OF I^{131}-LABELED γ-GLOBULIN FOR EACH SUBJECT. Plots indicate daily serum activity divided by the initial serum activity for purposes of comparison. M.F., G.P. and N.S.2 have amyloidosis. L.S., N.S., B.S. and J.P. have rheumatoid arthritis. J.G., F.W. and F.T. are normal controls.

lin used. The first group is composed of a patient (G.P.) with extensive primary amyloidosis but without renal involvement, and a patient (N.S.) with severe rheumatoid arthritis. The second group includes two subjects (B.S. and J.P.) with moderately severe rheumatoid arthritis; the third consists of a patient with severe active arthritis (L.S.) and a normal control (F.T.). The fourth group is composed of two control subjects (J. G. and F.W.), a patient with primary amyloidosis and nephrosis (M.F.), and finally, one of two patients with arthritis who had been studied in the first group and who had subsequently developed manifestations of amyloidosis with a nephrotic syndrome (N.S.).

The range of values for the half-time of globulin disappearance from the serum for the control subjects was 16.5 to 30 days. Two of the subjects with amyloidosis had values below this range and one of these, Patient N.S., studied before and after he developed clinical amyloidosis, showed a shortened half-time on both occasions. One patient with rheumatoid arthritis revealed a markedly shortened half-time of globulin disappearance. One subject with primary amyloidosis and three others with rheumatoid arthritis had disappearance times that were within the limits of the values for the control subjects.

In contrast to the values for serum disappearance, the values for the catabolic half-life of the injected protein show a wide variation. With three exceptions, however, the figures for serum disappearance and catabolic half-life are comparable. Possible explanations for the anomalous figures are discussed below. The values for distribution space as recorded in Table III do not show any significant tendencies.

TABLE III

Values for each subject of the parameters studied

Lot no.	Subject	Diagnosis	Serum half-time	Catabolic half-life	Distribution "space"
			days	*days*	*L*
1	G.P.	Amyloidosis	21	†	
	N.S.1*	Rheum. arthritis	14	†	
2	B.S.	Rheum. arthritis	19.5	12.0	9
	J.P.	Rheum. arthritis	17.5	14.5	4.5
3	L.S.	Rheum. arthritis	8.5	6.5	9
	F.T.	Control	30.	†	
4	N.S.2‡	Amyloidosis	11	9.5	8
	M.F.	Amyloidosis	4.5	3.5	6
	J.G.	Control	20	20	7.5
	F.W.	Control	16.5	16	3.5

* Patient with rheumatoid arthritis before clinical manifestations of amyloidosis.
† Determination unsatisfactory.
‡ Same patient after developing clinical amyloidosis.

DISCUSSION

The results of this study reveal three points of interest. First, they illustrate some of the complexities inherent in this method of study; second, they provide some interesting data with regard to amyloidosis; and third, they suggest a way in which rheumatoid factor might have biological activity.

The simplest parameter investigated was the rate of disappearance of the injected γ-globulin from the circulation. Although this figure is relatively free from inaccuracies of measurement, it reflects not only the rate of catabolism of the protein but also its distribution into different body compartments. For this reason the descriptive term "disappearance" has been used. It is evi-

TABLE IV

Rate of disappearance of I^{131}-labeled γ-globulin from the serum of normal humans as reported in other studies

Author	Subjects	Half-time serum disappearance	Remarks
		days	
Myant 1952 (14)	12 normal	6.0	10-day observation
Dixon and co-workers 1952 (15)	14 normal	13.1 ± 2.8	21-day observation
Eisenmenger and Slater 1953 (16)	8 normal	11.5 – 19	duration of study not stated
Havens and co-workers 1953 (17)	9 normal	10.6 – 16.6	40–60% of I^{131} excreted in first 4 days
Havens and co-workers 1954 (18)	19 normal	8–17	21-day observation
Wiener and Gordon 1957 (19)	1 agammaglobulinemia	35	unlabeled γ-globulin

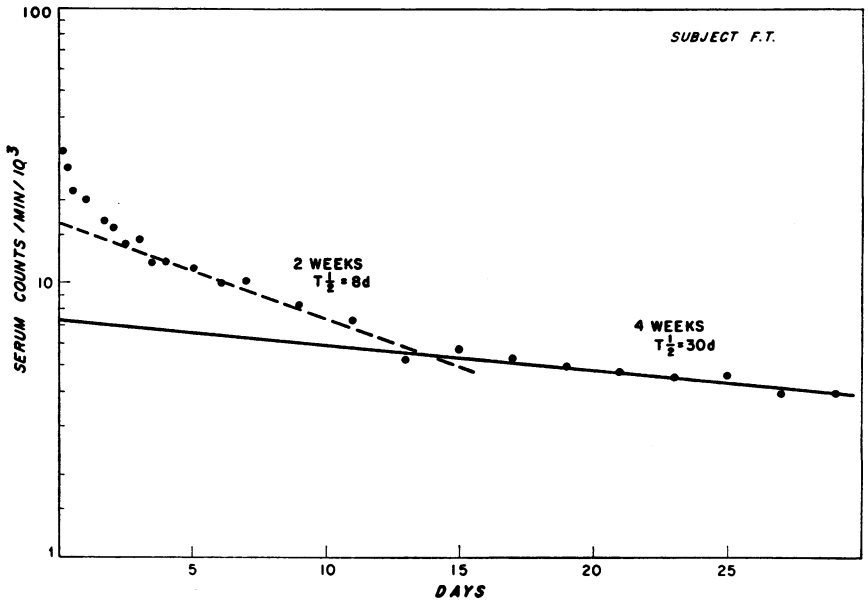

FIG. 3. EFFECT OF TERMINATING AN EXPERIMENT AT DAY 15. The solid line indicates the rate of disappearance as employed in this study. The dotted line indicates the apparent rate of disappearance had the study been terminated on Day 15.

dent that a wide range of values for this determination was encountered both in the normal subjects and in the patients studied.

A review of the literature reveals a similar spread of values for the half-time of serum globulin disappearance among normal subjects studied by different investigators (Table IV). It will be seen that the values for the control subjects in the present study are considerably longer than those recorded in Table IV. In some of these studies in which relatively short half-times of serum disappearance were found, the subjects were not followed for a period long enough to ensure that the curve plotted from the data approached a constant slope. Figure 3 illustrates the effect of a long as opposed to a short period of observation on the calculated rates of serum disappearance.

The possiblity that the labeling of proteins by radioactive iodine may alter their biological properties has been considered by several investigators (20, 21). In the preparation of the labeled protein used in this study, the ratio of moles of iodine to protein was calculated so that a maximal labeling of 3 atoms of iodine per molecule of γ-globulin was anticipated. McFarlane (22) has found that proteins lightly labeled with I^{131} have rates of catabolism similar to those of C^{14}-labeled material. Nevertheless, inferences about the metabolism of endogenous proteins from data obtained by the use of labeled protein must be guarded. The interpretation of tracer studies of this type is subject to a number of hazards. In addition to problems of sampling and measurement are those which involve the kinetics of distribution of the tracer material. These may be exceedingly complex. They have been discussed by Wrenshall (21) and by McFarlane (23). Despite these difficulties, some information of interest has been obtained.

The half-time of serum disappearance was accelerated in two of three patients with amyloidosis. One of these patients had "primary" disease; the other, pre-existing rheumatoid arthritis. Both, however, had clinical and laboratory evidence of the nephrotic syndrome. Since Gitlin and associates have shown that subjects with nephrosis from causes other than amyloidosis may have a shortened serum γ-globulin disappearance time (10), that state may have been the cause of the finding in these two patients. It is of interest that the half-time of γ-globulin disappearance was shortened in Subject N.S., both before and after he developed clinical evidence of amy-

loidosis. The pathogenesis of the shortened catabolic half-life of γ-globulin demonstrated in nephrotic subjects by Gitlin and co-workers is not known. The first study on Subject N.S. was performed 9 months before clinical evidence of nephrosis presented, and it is possible that a state of abnormal globulin metabolism was already present at that time. The fact that the third individual (G.P.), with very extensive primary amyloidosis but without nephrosis, showed a normal serum disappearance time suggests that the abnormal finding in the others was not due to amyloidosis per se.

Three of the four patients with rheumatoid arthritis showed a serum γ-globulin survival time within the normal range. It was definitely shortened in the fourth subject, L.S. Although this patient was, in general, comparable to the others, it is interesting that she is the only one with a high serum titer of rheumatoid factor. The rate of catabolism of the injected γ-globulin was similarly accelerated in this patient, showing that the rapid rate of disappearance from the serum was not due to sequestration of the protein in extravascular pools. Rheumatoid factor is capable of binding aggregated γ-globulin *in vitro* (24) and might conceivably do so when pooled γ-globulin is injected, thereby forming a complex which is more rapidly removed from the circulation and catabolized. Although, in accordance with the present evidence, this would be expected only if the injected γ-globulin were aggregated in some manner, the possible effects of the iodination procedure in this regard cannot be overlooked.[1]

The results obtained in this study may be compared with those of Vaughan and colleagues (25) who studied a group of patients with rheumatoid arthritis by a technique similar to that used here. Their results revealed a slightly but significantly more rapid rate of γ-globulin disappearance in the arthritics as compared with normal subjects. The duration of the individual studies was only 11 days, however, and for the reasons given above, this is probably too short to permit valid comparison. It is not known whether any of Vaughan's cases exhibited rheumatoid factor in high titer.

[1] Ultracentrifugal analysis showed that 94 per cent of the protein before iodination had a sedimentation coefficient of 7S.

The rate of γ-globulin catabolism agreed reasonably well with the half-time of serum disappearance in most instances in the present study. In several, however, it was very much accelerated. Technical difficulties were probably responsible for this discrepancy in three subjects—G.P., N.S.1 and F.T. Determination of the true rate of protein catabolism depends on the rate of excretion of the free radioactive marker in the urine and feces. Urine collections must be complete and continued until a constant rate of excretion is attained. In the three cases referred to above, the urine collections were unsatisfactory and the radioactivity became too low to measure accurately before either urine or serum activity had reached a constant rate of decline.

In a fourth study, B.S., in which urine collections were satisfactory, the rate of catabolism exceeded the rate of disappearance from the serum by a considerable factor. This finding can only be explained by the assumption that catabolism at a more rapid rate is taking place from some pool which is not in immediate exchange with the vascular one. While this would tend to delay the time required for the urine excretion curve to approach an asymptotic form, the difference in rates and the scatter of individual values tended to obscure this feature of the curve. It is therefore possible that the observed rate of catabolism is not the final rate which might have been attained had the experiment been prolonged.

Attempts to analyze the data from systems in which different rates of exchange occur between complexly related pools depend on certain assumptions which are difficult to prove; a fact that has limited the potential of this kind of study.

SUMMARY AND CONCLUSIONS

1. The serum survival time and catabolic half-life of intravenously injected I^{131}-labeled pooled human γ-globulin were studied in three patients with amyloidosis, four patients with rheumatoid arthritis and three normal controls.

2. The half-time of γ-globulin survival in the control subjects ranged from 16.5 to 30 days.

3. Two patients with amyloidosis, one primary and one secondary, both with the nephrotic syndrome, exhibited shortened serum half-times of 4.5 and 11 days, respectively. The serum half-

time of the latter patient, before the appearance of clinical amyloidosis, was 14 days.

4. One patient with primary amyloidosis but without nephrosis exhibited a half-time of serum γ-globulin disappearance of 21 days.

5. The half-time of γ-globulin disappearance in four patients with chronic active rheumatoid arthritis varied between 19.5 and 8.5 days. The lower figure was found in a patient having a high titer of rheumatoid factor. If this subject is excepted, the average half-time in three rheumatoid subjects is 17 days.

6. The catabolic half-life of the iodinated γ-globulin agreed in most instances with the serum half-time.

7. The calculated distribution space of the injected γ-globulin showed no consistent alteration in either amyloidosis or rheumatoid arthritis as compared with the control subjects.

8. Since the nephrotic syndrome from other causes may produce an accelerated catabolic half-life, a similar finding on our subjects cannot be ascribed to amyloidosis.

CASE HISTORIES

1. G.P. (M.G.H. no. 34 56 65). Two years prior to the study, this 55 year old bus driver noted the appearance of hemorrhagic blisters and red blotches on his eyelids, in his groins, and behind his ears. During the ensuing period he developed hypertrophic overgrowth of tissue in his auditory canals and papular masses on his tongue.

Physical examination was remarkable only in regard to the skin and tongue changes noted above. The liver and spleen were not palpable. Routine blood studies and urinalyses were negative. Biopsy of the papules on the tongue and ear and of the gingiva showed an infiltration of the skin with metachromatically staining, Congo red-positive material.

The patient died 8 months after study, probably as a result of cardiac arrhythmia. Autopsy disclosed extensive amyloid infiltration of the skin, tongue, thyroid and heart. There was no involvement of liver, spleen or kidneys.

2. N.S. (M.G.H. no. 20 57 55). This 37 year old man had experienced severe crippling rheumatoid arthritis since he was 14 years of age, and from the age of 26 was confined to bed and wheelchair.

At the time of the first study in July 1957, physical examination revealed severe deformities of hands and feet and greatly limited motion of temperomandibular, hip, knee and ankle joints, and spine. The liver and spleen were not palpable.

During the ensuing year he had a mild exacerbation of his arthritis, developed 2+ albuminuria for the first time, his liver became enlarged, and a Congo red test showed only 12 per cent retention in the serum. Gingival and renal biopsies showed changes consistent with a diagnosis of amyloidosis.

The patient developed slowly progressive uremia and died in June 1959. An autopsy revealed amyloidosis involving primarily the liver, spleen and kidneys.

3. B.S. (M.G.H. no. 52 60 05). This 60 year old woman had rheumatoid arthritis of 34 years' duration. Its course was characterized by many exacerbations and remissions initially involving the peripheral joints and, at a later date, the spine. The arthritis was accompanied by psoriasis and 3 years before study mild diabetes was discovered. She never developed rheumatoid nodules.

Physical examination revealed moderate limitation of most peripheral joints, hips and spine. The liver and spleen were not palpable. Routine blood and urine studies were not remarkable. A gingival biopsy was negative for amyloid. Congo red tests have been negative on several occasions.

4. J.P. (M.G.H. no. 78 72 05). This 61 year old woman had rheumatoid arthritis of 9 years' duration. The hands, wrists, elbows, knees, feet, temperomandibular joints and cervical spine became involved early in her course. Although the disease gradually became less active, a complete remission was never achieved. Typical rheumatoid subcutaneous nodules were present.

Physical examination disclosed moderately advanced stigmata of rheumatoid arthritis. Laboratory studies revealed normal blood indices. Urinalysis was negative except for a trace of albumin. A Congo red test showed 67 per cent retention in the serum.

5. L.S. (M.G.H. no. 74 53 16). The patient was a 62 year old housewife with rheumatoid arthritis of 8 years' duration. Starting in her hands and feet, the articular involvement spread to the knees, shoulders, cervical spine and temperomandibular joints. During two severe exacerbations she received corticosteroid therapy. Her disease was only moderately active at the time of the study and the corticosteroid therapy had been stopped 4 months previously.

Examination revealed moderately reduced mobility of the above-mentioned joints with slight periarticular swelling. Subcutaneous nodules were not present. Blood studies revealed a moderate normochromic anemia with a hemoglobin of 11.4 g. Urinalysis showed 2+ albumin and 20 to 30 white blood cells per high-power field. A Congo red test was negative, 62 per cent of the dye remaining in the serum. The latex fixation test was positive in a dilution of serum greater than 1:2,560.

6. F.T. (M.G.H. no. 67 96 90). The patient was a 63 year old laborer who had been admitted to the hospital 1 month previously because of an active duodenal ulcer and iron-deficiency anemia. Past history was negative except for pneumonia 10 years previously and chronic alcoholism.

Physical examination was unremarkable. Laboratory studies revealed no evidence of renal or hepatic dysfunc-

tion. Total serum protein was 6.6 g; albumin, 4.5 g; and globulin, 2.1 g per cent.

7. M.F. (M.G.H. no. 98 66 63). This 50 year old truck driver noted the insidious onset of edema of the feet, ankles, face and hands 1 year before the time of the study. There had been no preceding respiratory or other infections. A urinalysis 6 months prior to the onset of symptoms was normal.

The patient was admitted to the hospital 6 months before the present study. The physical examination at that time was negative except for moderate edema of the feet and hands. Routine blood studies were normal. The urinary sediment contained moderate numbers of white cells and the 24-hour protein excretion varied from 10 to 60 g. Urine culture was sterile. The renal creatinine clearance was 137 L per 24 hours.

A Congo red test revealed no retention of dye in the serum and a renal biopsy showed massive amyloidosis. A trial of prednisone therapy on two occasions produced no improvement and the drug was discontinued 3 months before the time of study.

At the commencement of this study the physical findings were essentially as described above except that the liver and spleen were just palpable. Urinary protein excretion was 8.5 and 9.8 g per 24 hours on two occasions. Sulfobromophthalein retention was 22 per cent at 45 minutes. No medications were given during the study.

8. J.G. (M.G.H. no. 27 41 01). This 48 year old man had contracted poliomyelitis at the age of 6 months. As a result he had a flail right shoulder, weak right elbow and almost flail feet bilaterally. Due to falls over the 20 years prior to admission, he sustained fractures of the right femur, left hip and left tibia, the last occurring 6 months before study. His past history was otherwise negative. At the time of study, he was up in a wheelchair undergoing a physical therapy program. Physical examination, except as above, and routine laboratory studies were entirely negative.

9. F.W. (M.G.H. no. 84 89 06). This 41 year old itinerant worker was admitted to the hospital specifically for study as a control subject. He had been well all his life except for moderate alcoholism since the age of 15.

On physical examination he appeared normal. There were no stigmata of cirrhosis. Routine blood and urine studies were negative. Liver function studies were normal.

APPENDIX

In two cases (N.S. 2 and M.F.) the excretion of protein-bound radioactivity in the urine was sufficiently great to permit accurate counting, and the half-times for serum activity disappearance were corrected by the following method.

Assumptions involved in the calculations were: *a*) intercompartmental mixing of the labeled protein is complete and takes place at rates greatly in excess of the combined rates of excretion of protein and nonprotein activity into the urine, and *b*) the concentration of free radioiodide in the blood is negligible. Therefore,

$$Ct = Co\, e^{-[k_1+k_2]t}$$

where Ct = the observed serum activity at any time t after mixing is complete; Co = the serum activity at $t = 0$; k_1 = the rate constant for the loss of protein-bound activity into the urine; k_2 = the rate constant for the loss of free radioiodide into the urine.

The values for each of these constants may be determined by the relation

$$k_1/k_1 + k_2 = \text{urine protein-bound activity/total urine activity}$$

for any given period after mixing is complete.

From these data k_2 may be determined and a corrected half-time of disappearance of iodinated γ-globulin from the serum computed.

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