JCI The Journal of Clinical Investigation

Behavior in vivo of normal and dysfunctional C1 inhibitor in normal subjects and patients with hereditary angioneurotic edema.

M Quastel, R Harrison, M Cicardi, C A Alper, F S Rosen

J Clin Invest. 1983;71(4):1041-1046. https://doi.org/10.1172/JCI110831.

Research Article

The metabolism of normal C1 inhibitor and two dysfunctional C1 inhibitors (Ta and WeI) was studied in 10 normal subjects and 8 patients with hereditary angioneurotic edema (HANE), 4 with low antigen concentration (type 1) and 4 with dysfunctional protein (type 2). The fractional catabolic rate of the normal C1 inhibitor in normal subjects was 0.025 of the plasma pool/hour, whereas in HANE subjects it was significantly elevated at 0.035 of the plasma pool/hour. The synthesis of normal C1 inhibitor was decreased in patients with type 1 HANE (0.087 mg/ kg per h compared with 0.218 mg/kg per h). The fractional catabolic rate of dysfunctional protein WeI was similar to normal and showed a slightly accelerated catabolism in patients with HANE, whereas the dysfunctional protein Ta had a strikingly decreased fractional catabolic rate in normals and subjects with HANE. The present study is compatible with reduced C1 inhibitor synthesis in patients with type 1 HANE consistent with a single functional C1 inhibitor gene. The lower than anticipated levels of C1 inhibitor in HANE type 1 appears to result from (a) the single functional gene and (b) increased catabolism of the protein, perhaps related to activation of C1 or other proteases.

Behavior In Vivo of Normal and Dysfunctional C1 Inhibitor in Normal Subjects and Patients with Hereditary Angioneurotic Edema

MICHAEL QUASTEL, RICHARD HARRISON, MARCO CICARDI, CHESTER A. ALPER, and FRED S. ROSEN, *Department of Pediatrics, Harvard Medical School, the Center for Blood Research, Department of Medicine, Children's Hospital Medical Center, Boston, Massachusetts 02115; Department of Medicine, Università di Milano, Ospedale San Paolo, Milan, Italy*

ABSTRACT The metabolism of normal C1 inhibitor and two dysfunctional C1 inhibitors (Ta and Wel) was studied in 10 normal subjects and 8 patients with hereditary angioneurotic edema (HANE), 4 with low antigen concentration (type 1) and 4 with dysfunctional protein (type 2). The fractional catabolic rate of the normal C1 inhibitor in normal subjects was 0.025 of the plasma pool/hour, whereas in HANE subjects it was significantly elevated at 0.035 of the plasma pool/hour. The synthesis of normal C1 inhibitor was decreased in patients with type 1 HANE (0.087 mg/kg per h compared with 0.218 mg/kg per h). The fractional catabolic rate of dysfunctional protein Wel was similar to normal and showed a slightly accelerated catabolism in patients with HANE, whereas the dysfunctional protein Ta had a strikingly decreased fractional catabolic rate in normals and subjects with HANE. The present study is compatible with reduced C1 inhibitor synthesis in patients with type 1 HANE consistent with a single functional C1 inhibitor gene. The lower than anticipated levels of C1 inhibitor in HANE type 1 appears to result from (*a*) the single functional gene and (*b*) increased catabolism of the protein, perhaps related to activation of C1 or other proteases.

INTRODUCTION

Hereditary angioneurotic edema (HANE)[1] results from a defect in the C1 inhibitor (C1 INH) (1, 2); susceptibility to attacks of angioedema is inherited as an autosomal dominant trait. Most patients with HANE have decreased levels of apparently normal C1 INH (type 1). Patients in ~15% of affected kindred, however, have normal or elevated serum concentrations of dysfunctional C1 INH (type 2) (3). A number of functionally inactive molecular variants have been reported (4–6).

In patients with reduced C1 INH serum concentrations, serum levels are 5–31% of normal rather than 50% of normal as expected from the presence of one normal gene for C1 INH (4). Moreover, little or no normal C1 INH is detected in serum of patients with dysfunctional protein when the former can be distinguished from the latter (4). It may be that increased catabolism of normal C1 INH, perhaps related to C1 activation, accounts for these observations.

In the present study, we have examined the metabolic behavior of radiolabeled normal C1 INH and of two different dysfunctional proteins in normal subjects and in patients with type 1 or type 2 HANE.

METHODS

Protein purification. Normal C1 INH and the dysfunctional C1INH proteins Ta and Wel (4) were prepared from fresh or fresh frozen plasma. All units were tested and found negative by radioimmunoassay for HB_sAg. The details of the method of purification are presented elsewhere.[2] In brief, fibrinogen and other aggregated material was precipitated from ACD plasma (containing EDTA and benzamidine) with polyethylene glycol 4000. Plasmin and plasminogen were then removed from the supernatant by passage over lysine-Sepharose (Pharmacia Fine Chemicals, Div. Pharmacia, Inc., Piscataway, NJ). The eluate was subsequently fractionated on diethylaminoethyl Sephadex A-50 (Pharmacia Fine

Address reprint requests to Dr. Rosen.

Received for publication 20 August 1982 and in revised form 15 December 1982.

[1] *Abbreviations used in this paper:* C1 INH, C1 inhibitor; E/P, extravascular/plasma; FCR, fractional catabolic rate; HANE, hereditary angioneurotic edema.

[2] Harrison, R. Submitted for publication.

Chemicals), and CĪ INH-containing fractions were gel filtered on Sephadex G-150 superfine (Pharmacia Fine Chemicals). Final purification was achieved on hydroxylapatite (Bio-Rad Laboratories, Richmond, CA). The purified CĪ INH was >95% pure as judged by SDS polyacrylamide gel electrophoresis. Recovery of normal CĪ INH was 70–75% calculated as protein but >100% as functional activity in a hemolytic assay (7). During purification, the dysfunctional CĪ INH proteins fractionated similarly to normal CĪ INH and the yields were also 70–75%.

Radiolabeling. Purified proteins were labeled with ^{125}I or ^{131}I (New England Nuclear, Boston, MA) by the iodine monochloride technique (8). Free radioactivity was removed by gel filtration on a PD-10 Sephadex G-25M column (Pharmacia Fine Chemicals). Human albumin was added to 5 mg/ml, and the labeled protein solution was dialyzed against phosphate-buffered saline at pH 7.4 and sterilized by Millipore filtration (Millipore Corp., Bedford, MA). Each subject received 1–3 μCi of ^{125}I or ^{131}I-labeled normal or dysfunctional protein intravenously. In some cases, both normal (^{131}I) and dysfunctional (^{125}I) CĪ INH were given simultaneously from the same syringe. The specific functional activity of radiolabeled normal CĪ INH was 6.75×10^{15} effective molecules/mg CĪ INH, the same as the purified material before radiolabeling (6.74×10^{15} effective molecules/mg CĪ INH). Moreover, on incubation with CIs in molar excess, >95% of the radiolabeled CĪ INH formed a covalently bonded complex (9).

Subjects. There were 10 healthy control subjects ranging in age from 25 to 47 yr. Eight patients with HANE were studied, four with type 1 and four with type 2. Two patients with low CĪ INH were sustaining mild attacks of angioedema during the study. All subjects received 10 drops of a saturated solution of potassium iodide by mouth twice daily to block uptake of labeled iodine by the thyroid and ensure complete urinary excretion of radioactive iodine released by catabolism.

Collection and treatment of samples. Blood samples were collected into EDTA and were centrifuged at ~2,000 rpm for 10 min. 2 ml of plasma were analyzed for ^{125}I and/or ^{131}I radioactivity in a gamma scintillation counter. Samples were collected 10 min after injection, and then at intervals of 0.5, 1, 2, 4, 8, 24 h and twice a day thereafter for 5–8 d.

Urine was collected throughout the period of study. Aliquots of 2 ml were assayed for radioactivity under the same geometric conditions as used for the plasma samples.

Analysis of data. The radioactivity of plasma samples was expressed as decimal fraction of that in the 10-min sample and plotted on semilogarithmic paper. The resulting curve was resolved manually into exponentials for analysis by the Matthews' method (10). In some cases, the method of Nosslin (11) was also used. By these methods, the fractional catabolic rate (FCR) as decimal fraction of the plasma pool per h, the synthesis rate as milligrams per kilogram per hour and the extravascular/plasma (E/P) pool ratio were calculated. For the synthesis rate, CĪ INH concentration in serum was determined by electroimmunoassay (12) and the plasma volume was assessed by isotope dilution in the 10-min sample. The synthesis rate was then calculated by multiplying FCR by plasma pool and dividing by body weight.

RESULTS

Fig. 1 and Table I present the results of the study of the metabolism of radiolabeled normal CĪ INH in nine control subjects. Fig. 2 and Table II provide the same kind of information for five patients with HANE.

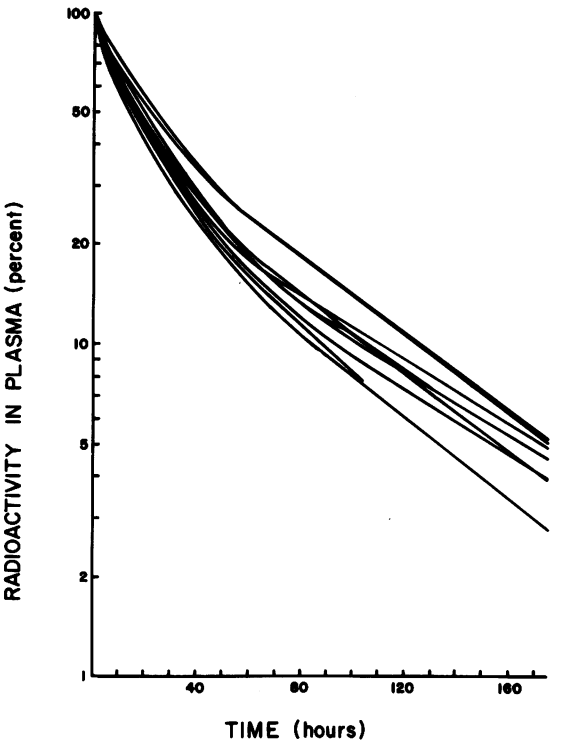

FIGURE 1 The plasma radioactivity curves of CĪ INH labeled with radioactive iodine in nine normal subjects.

The FCR of radiolabeled normal CĪ INH in normal subjects was 0.025 of the plasma pool/h±0.002, calculated by either the Matthews or Nosslin method and the E/P ratio was 0.60±0.06. In the five patients, the FCR of CĪ INH was significantly elevated ($P < 0.001$ at 0.035±0.001 [M] or 0.038±0.002 [N]), as was the E/P ratio at 1.26±0.13 ($P < 0.001$). The disappearance curves of the labeled normal protein were distinctly different in the normal subjects compared with the patients, as seen by comparing Fig. 1 with Fig. 2. Most of this difference was in the early portions of the curves. The slopes of the final exponentials in patients were, if anything, slightly more shallow than those of healthy subjects. There was no apparent difference in the metabolic behavior of the labeled normal CĪ INH in patients with low protein concentration and those with dysfunctional proteins and in the two patients having angioedema at the time of the study.

The synthesis of normal CĪ INH in patients with low protein concentration was decreased at 0.087 and 0.07 mg/kg per h compared with a rate in normal subjects of 0.218±0.08 mg/kg per h ($P < 0.001$). Because it is not possible to estimate accurately the concentration of normal CĪ INH in the plasma of patients

TABLE I
Metabolism of C1̄ INH in Normal Subjects

Subject	Sex	Age	C1̄ INH	FCR (Fraction plasma pool/h) Matthews	FCR (Fraction plasma pool/h) Nosslin	E/P	Synthesis rate
			mg/dl				*mg/kg per h*
R.H.	M	32	17.4	0.027	0.030	0.81	0.23
J.C.	M	30	16.4	0.031	ND	0.59	0.26
A.F.	M	28	18.5	0.020	0.017	0.29	0.23
J.A.	F	30	23.1	0.025	0.030	0.76	0.26
M.S.	F	32	18.5	0.029	0.030	0.64	0.27
J.N.	F	25	19.5	0.025	ND	0.56	0.18
L.T.	F	25	19.1	0.020	ND	0.63	0.20
K.C.	F	22	15.9	0.020	0.022	0.41	0.15
D.S.	F	47	19.5	0.026	0.021	0.70	0.18
Mean±SEM			18.7±0.7	0.025±0.001	0.025±0.002	0.60±0.06	0.22±0.08

with dysfunctional C1̄ INH no attempt to determine synthesis rates for normal C1̄ INH in these patients was made.

Fig. 3 and Table III depict results from the study of radiolabeled dysfunctional proteins Ta and WeI in normal subjects and patients with HANE. It is clear that the two proteins differed in their metabolic behavior from one another. The plasma disappearance curves, FCR and E/P ratios of WeI dysfunctional protein in normal subjects (Fig. 3 and Table III) were similar to those observed for normal C1̄ INH in normal subjects.

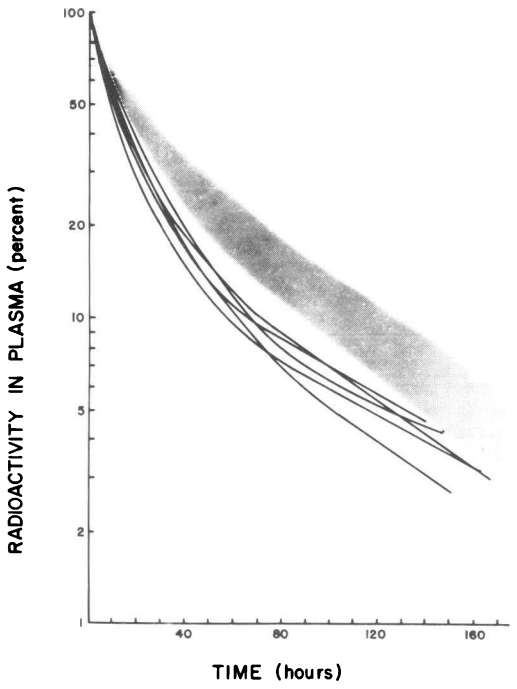

FIGURE 2 The plasma radioactivity curves obtained in five studies of patients with types 1 and 2 HANE. The stippled area is the range of normal curves shown in Fig. 1.

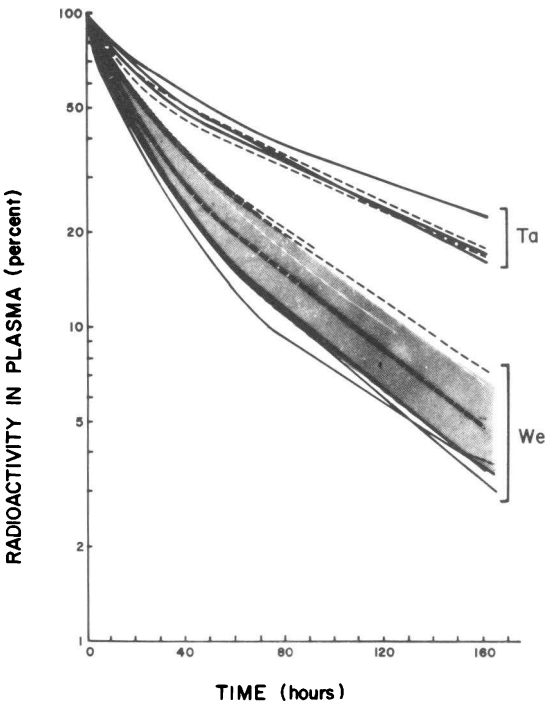

FIGURE 3 The plasma radioactivity curves of the dysfunctional C1̄ INH Ta and WeI obtained in five studies of normal subjects (- - -) and in six studies in patients with types 1 and 2 HANE (——).

TABLE II
Metabolism of Normal CĪ INH in Patients with HANE

Code	Sex	Age	HANE	CĪ INH	FCR Fraction plasma pool/h Matthews	FCR Fraction plasma pool/h Nosslin	E/P	Synthesis rate
				mg/dl				*mg/kg/h*
M.N.	M	19	Type 2	16.1	0.039	0.040	0.87	ND
W.J.	M	62	Type 1	4.8	0.036	0.034	1.27	0.087
R.K.	M	17	Type 1	4.8	0.035	0.035	1.40	0.070
M.M.	M	43	Type 2	14.9	0.031	0.046	1.67	ND
D.M.	M	33	Type 2	15.9	0.036	0.034	1.11	ND
Mean±SEM					0.035±0.001	0.038±0.002	1.26±0.13	

The metabolic behavior of the Ta dysfunctional protein on the other hand, differed from that of normal and WeI CĪ INH proteins, as can be seen from Fig. 3 and Table III. It had a strikingly slower plasma disappearance than the latter two proteins, and did not show accelerated clearance in patients with HANE. The FCR of Ta protein in both groups of subjects, at 0.009 (N) to 0.011 (M), was less than half that of the normal or WeI proteins in normal subjects. The E/P ratio of Ta protein, at an average of 0.31, was also about half that of the normal CĪ INH and WeI protein in normal subjects. It was similarly low in patients with HANE.

In these studies there appeared to be a direct relationship between FCR and E/P ratio. From Fig. 4 it is seen that this relationship, although striking, was not strictly linear.

DISCUSSION

In most inherited deficiency states of plasma proteins, including components of complement, heterozygous carriers have ~50% of the normal serum level. This is consistent with the presence of one normal and one silent, or nearly silent, gene. In contrast, patients with type 1 HANE are heterozygous and yet have, on average, only 17% of the normal serum concentration of apparently normal CĪ inhibitor (with a range of 5–31%) (4). Patients with the dysfunctional protein forms of HANE are also heterozygotes and yet have little or no detectable normal CĪ INH (4).

One possible explanation for these findings is that at half-normal serum concentration of CĪ INH, there is activation of the early classical complement pathway and/or other systems in which this protein acts as an

TABLE III
Metabolism of Dysfunctional CĪ INH in Normal Subjects and HANE Patients

Dysfunctional status of CĪ INH	Subject	Code	Age	Sex	FCR Fraction plasma pool/h Matthews	FCR Fraction plasma pool/h Nosslin	E/P
Ta	Control	J.U.	25	F	0.012	0.008	0.31
Ta	Control	D.S.	47	F	0.011	0.009	0.24
Ta	HANE type 2	I.C.	46	M	0.009	0.009	0.36
Ta	HANE type 2	P.M.	33	M	0.011	0.008	0.39
Ta	HANE type 2	M.N.	19	M	0.012	0.008	0.23
We	Control	A.F.	28	M	0.019	0.018	0.39
We	Control	J.F.	28	M	0.020	0.020	0.44
We	Control	K.C.	22	F	0.025	0.022	0.50
We	HANE type 1	W.J.	62	M	0.029	0.030	0.70
We	HANE type 2	M.M.	43	M	0.027	0.031	1.16
We	HANE type 2	D.M.	33	M	0.031	0.028	0.68
We	HANE type 1	F.C.	30	M	0.031	0.031	0.73

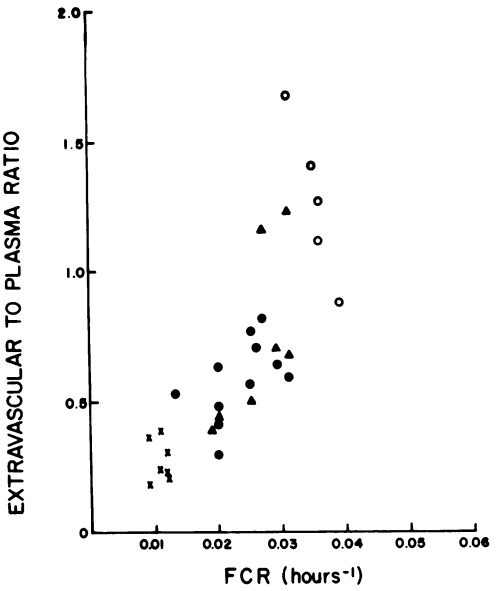

FIGURE 4 The relationship between FCR and E/P ratio. X Ta protein in normal and HANE subjects; ●, normal C1 inhibitor in normal subjects; ▲, Wel protein in normal and HANE subjects; and O, normal C1 inhibitor in HANE subjects.

inhibitor. This in turn could lead to consumption of the normal CĪ INH, with the result that its concentration would fall below 50% of normal.

The present studies show clearly that the actual situation is more complex and that both increased catabolism, predicted by the mechanism postulated above, and decreased synthesis of normal CĪ INH contribute to the low serum concentration of this protein in all forms of HANE. Although this is most evident in patients with type 1 form of the disease because the concentration of normal CĪ INH can be measured precisely, it must also be true of the dysfunctional protein form of HANE, because normal CĪ INH is probably in even lower concentration in the plasma (4). In earlier studies of patients with type 1 HANE (13), we found that liver cells contained no CĪ INH detectable by fluorescent antiserum, whereas liver from normal subjects contains 5–10% of such hepatocytes. These findings were interpreted as showing decreased synthesis of CĪ INH in these patients, confirmed by the present studies. Neither the previous studies nor the present experiments exclude the possibility that synthesis is reduced by the 50% predicted by the presence of only a single functional gene.

Previous studies (4) had revealed extensive genetic heterogeneity among patients with type 2 HANE. At least four distinct dysfunctional CĪ INH were distinguishable by agarose gel electrophoresis: (*a*) normal concentration, normal electrophoretic mobility, Wel; (*b*) normal concentration, moderately increased mobility, Za; (*c*) normal concentration, markedly increased mobility, Ta: and (*d*) increased concentration with albumin complexes and moderately increased mobility, Da. In addition, differences were noted in the ability of different dysfunctional CĪ INH to bind CĪ and to block the esterolytic activity of CĪ (all fail to block the C4 inactivating activity of CĪ).

Wel protein has the same or similar electrophoretic mobility as normal CĪ INH but has little ability to bind CĪ in vitro. In the present study, it behaved metabolically very much like normal CĪ INH. The Ta protein has the largest increase in electrophoretic mobility of known dysfunctional CĪ INH, and recent evidence (unpublished observations) suggests that it is 4,000 D larger than the normal counterpart. The difference resides in the CNBr-2 fragment and is not due to carbohydrate but rather to an insertion of amino acids (14). Nevertheless, it binds CĪ although probably to a reduced extent. Its metabolic behavior was distinctly aberrant in that its FCR was markedly reduced compared with normal CĪ INH, and there was no increase in its catabolism in patients with HANE. There is no obvious explanation for these phenomena, but they suggest that there may be a structural feature on the CĪ INH molecule involved in its catabolism, and that this feature is absent or altered on the Ta protein.

It is difficult to interpret the direct relationship between FCR and E/P ratio found in the present studies. A similar relationship appears to hold for C3 (15) and properdin (16). It could be methodological and related to the method of analysis although it does not occur in general with other, noncomplement proteins (17). A possible interpretation is that there is increased reversible removal of labeled protein from the plasma pool. In the case of functional CĪ INH this could result from noncovalent complex formation with tissue-bound protease(s). It could also be argued that the increased FCR and E/P ratio are both the results of HANE, the E/P ratio increase reflecting the increased vascular permeability characteristic of the disease. Against this possibility is the lack of increase in E/P for the Ta protein studied in patients with HANE, in whom simultaneously injected normal CĪ INH showed increased E/P ratios.

Previous studies of the metabolic behavior of normal CĪ INH have been problematic because of the difficulty in purifying this protein in a native and functional state in the past. Brackertz et al. (18) studied normal CĪ inhibitor in three HANE patients and three normal subjects and found no apparent differences among them. Their CĪ INH preparation had only half of the anticipated functional activity.

ACKNOWLEDGMENTS

This work was supported by grants from the Birth Defects Foundation-March of Dimes and grants RR128, AI 05877, AI 14157, and AI 15033 of the U. S. Public Health Service.

REFERENCES

1. Donaldson, V. H., and R. R. Evans. 1963. A biochemical abnormality in hereditary angioneurotic edema. *Am. J. Med.* **35:** 37–44.
2. Landerman, N. S., M. E. Webster, E. L. Becker, and H. E. Ratcliffe. 1962. Hereditary angioneurotic edema. II. Deficiency of inhibitor for serum globulin permeability factor and/or plasma kallikrein. *J. Allergy.* **33:** 330–341.
3. Rosen, F. S., P. Charache, J. Pensky, and V. H. Donaldson. 1965. Hereditary angioneurotic edema: two genetic variants. *Science (Wash. DC).* **148:** 957.
4. Rosen, F. S., C. A. Alper, J. Pensky, M. R. Klemperer, and V. H. Donaldson. 1971. Genetically determined heterogeneity of the C1 esterase inhibitor in patients with hereditary angioneurotic edema. *J. Clin. Invest.* **50:** 2143–2149.
5. Harpel, P. C., T. E. Hugli, and N. R. Cooper. 1975. Studies on human plasma C$\bar{1}$ inactivator-enzyme interactions. II. Structural features of an abnormal C$\bar{1}$ inactivator from a kindred with hereditary angioneurotic edema. *J. Clin. Invest.* **55:** 605–611.
6. Laurell, A.-B., J. Lindegren, I. Malmros, and H. Mårtensson. 1969. Enzymatic and immunochemical estimation of C1 esterase inhibitor in sera from patients with hereditary angioneurotic edema. *Scand. J. Clin. Lab. Invest.* **24:** 221–225.
7. Gigli, I., S. Ruddy, and K. F. Austen. 1968. The stoichiometric measurement of the serum inhibitor of the first component of complement by the inhibition of immune hemolysis. *J. Immunol.* **100:** 1154–1164.
8. McFarlane, A. S. 1958. Efficient trace-labelling of proteins with iodine. *Nature (Lond.).* **182:** 53.
9. Harpel, P. C., and N. R. Cooper. 1975. Studies on human plasma C$\bar{1}$ inactivator-enzyme interactions. I. Mechanisms of interaction with C$\bar{1}$s, plasmin, and trypsin. *J. Clin. Invest.* **55:** 593–604.
10. Matthews, C. M. E. 1957. The theory of tracer experiments with ^{131}I labeled plasma proteins. *Phys. Med. Biol.* **2:** 36–53.
11. Nosslin, B. 1973. Analyses of disappearance time-curves after single injection of labeled proteins. *Ciba Found. Symp.* **9:** 113–128.
12. Laurell, C-B. 1966. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Anal. Biochem.* **15:** 45–52.
13. Johnson, A. M., C. A. Alper, F. S. Rosen, and J. M. Craig. 1971. C1-inhibitor: Evidence for decreased hepatic synthesis in hereditary angioneurotic edema. *Science (Wash. DC).* **173:** 5553–5554.
14. Harrison, R. A., and F. S. Rosen. 1982. Structural characterization of C1-esterase inhibitor and comparison with dysfunctional proteins from individuals with HANE. *Mol. Immunol.* **19:** 1374.
15. Alper, C. A., and F. S. Rosen. 1967. Studies of the in vivo behavior of human C'3 in normal subjects and patients. *J. Clin. Invest.* **46:** 2021–2034.
16. Ziegler, J. B., F. S. Rosen, C. A. Alper, W. Grupe, and I. H. Lepow. 1975. Metabolism of properdin in normal subjects and patients with renal disease. *J. Clin. Invest.* **56:** 761–767.
17. Alper, C. A., T. Freeman, and J. Waldenström. 1963. The metabolism of gamma globulins in myeloma and allied conditions. *J. Clin. Invest.* **42:** 1858–1868.
18. Brackertz, D., E. Isler, and F. Kueppers. 1975. Half life of C$\bar{1}$ INH in hereditary angioneurotic edema (HAE). *Clin. Allegy.* **1:** 89–94.