
Studies on the Metabolism of Fibrinogen in Two Patients with Congenital Afibrinogenemia

By DAVID GITLIN, M.D.* AND WAYNE H. BATES, M.D.

A FIBRINOGENEMIA, a hemorrhagic diathesis associated with a clotting time of infinity and presumably due to the absence of fibrinogen,¹ is one of a group of diseases manifested by the deficiency of an individual plasma protein. Since the absence of circulating fibrinogen may result from errors either in synthesis or in degradation, we have studied two children with congenital afibrinogenemia in an effort to determine the nature of the metabolic defect and to obtain further information concerning the extravascular protein pool.²

Because of uncertainties concerning the accuracy of fibrinogen determinations obtained by clotting procedures,^{3, 4} particularly at low levels of fibrinogen, an immunochemical method was employed in this study to estimate fibrinogen specifically, quantitatively, and independently of the other factors involved in the clotting mechanism. The data suggest that these patients are unable to synthesize significant amounts of fibrinogen. The data also emphasize the role of the extravascular protein pool in plasma protein therapy.

MATERIALS AND METHODS

Two patients with congenital afibrinogenemia, already presented in detail clinically,⁵ were selected for study. Patient C. M., a boy, age 5 years and 3 months, was given 1.2 Gm. of human fibrinogen, in terms of clottable protein (*vide infra*), intravenously as fraction I,⁶ preparation IFF-43A, dissolved in 150 ml. of 5 per cent dextrose. This patient had received a similar injection of fraction I three weeks earlier, but his coagulation studies (*vide infra*) had returned to preinfusion values before the infusion studied here was given. Patient E. J., a boy, age 9 years and 7 months, was given 1.2 Gm. of human fibrinogen, in terms of clottable protein, sterilized by ultraviolet irradiation, preparation EX 309, by intravenous infusion in 100 ml. of 5 per cent dextrose; he had not received any human blood products in any form for at least six months prior to this infusion. Both infusions were given over a period of 15 minutes. Samples of blood were taken before and after infusion, the end of the infusion being taken as "zero time". For fibrinogen analysis, 9 ml. of blood were mixed carefully with 1 ml. of 2.5 per cent sodium citrate for C. M. and with 25 mg. solid sodium citrate for E. J., centrifuged at 2000 R.P.M. 0 C. for 10 minutes and the supernatant plasma then

From the Department of Pediatrics, Harvard Medical School, and the Children's Medical Center, Boston, Mass.

Submitted December 10, 1952; accepted for publication January 31, 1953.

Supported by a grant (RG-346) from the National Institutes of Health, United States Public Health Service, and a grant from the Variety Club of New England.

* Work completed while under tenure of a post-doctoral research fellowship, National Institutes of Health, United States Public Health Service.

The authors gratefully acknowledge their indebtedness to Dr. Charles A. Janeway for his aid in the preparation of this manuscript and to Mrs. Gloria Reed for her technical assistance.

centrifuged for 15 minutes at 3500 R.P.M. at 0 C. Appropriate dilutions of plasma in 0.15 M. NaCl were reacted with antiserum within an hour of collection of the blood sample.

The preparation of rabbit antisera against human fibrinogen has been described.² Rabbits were injected subcutaneously with an aluminum hydroxide suspension of carefully prepared fibrin. Such antisera gave typical rabbit precipitation curves with fibrinogen; antibodies against any of the components of normal human serum could not be detected. Heparin was added to the antisera, making a final concentration of 0.3 mg. heparin per ml. antiserum. When such antisera were mixed with equal volumes of undiluted, citrated normal rabbit plasma, no clotting was visible in 24 hours. In every instance in the reaction between the rabbit antisera and human plasma, the specific precipitate was flocculent and no trace of fibrin clot or net was found.

The antisera were standardized employing appropriate dilutions of samples of the fibrinogen solutions given to these patients and appropriate dilutions of plasma obtained from these patients 15 minutes after fibrinogen infusion. The fibrinogen content of the infusion solutions was determined gravimetrically as fibrin, employing the following conditions for clotting⁴: Human thrombin* 0.1 units/cc., fibrinogen approximately 1.0 Gm./liter, temperature 25 C. After mixing antigen and antiserum, the mixtures were incubated at 37 C. for 1 hour and then at 2 to 5 C. overnight; the precipitates were washed and measured spectrophotometrically.⁷ After standardization, the antisera were stored at -30 C. in lots of 6 cc. and a fresh lot of antiserum used for each series of determinations. This procedure avoided freezing or prolonged storage of the plasma samples with resultant precipitation of fibrin with time and avoided complete restandardization of antiserum for a single fibrinogen determination. To minimize errors due to possible immunochemical heterogeneity of fibrinogen, the following precautions were taken:

1. Antiserum was standardized with plasma obtained from the patient 15 minutes after fibrinogen infusion and was used for estimating fibrinogen only in plasmas from that patient.

2. A minimum of three different plasma concentrations were run for each blood sample to establish that, if immunochemically heterogeneous, the fibrinogen components remained in fixed ratio while metabolized.⁵

3. The results were expressed in terms relative to the concentration of administered fibrinogen found in the patient's plasma 15 minutes after infusion.

Electrophoreses were done at pH 8.6, sodium diethylbarbiturate buffer, $\Gamma/2 = 0.1$. Clotting times and sedimentation rates were done by conventional methods.^{9, 10}

About four months after his last fibrinogen infusion, muscle and skin biopsies of the right forearm were performed on E. J. under general anesthesia (ether). Immediately after operation, 1.2 Gm. fibrinogen (fraction I) were given intravenously and 24 hours later, muscle and skin biopsies of the right arm were performed, again under general anesthesia (cyclopropane). These tissues were sectioned and treated for the detection of fibrinogen, plasma albumin, γ -globulin and β -metal combining globulin with fluorescein-labelled antibodies as described.^{11, 2} The individual plasma protein was traced by the resulting specific fluorescence in the tissue sections when studied under the ultraviolet microscope.

RESULTS

Although preinfusion blood samples taken without anticoagulants showed no visible evidence of clotting after 24 hours, small amounts of fibrinogen (0.8 to 1.2 mg. per cent) were detected in the corresponding plasmas immunochemically; addition of thrombin to preinfusion plasma resulted in a slight increase in turbidity without formation of a fibrin net. The rate of disappearance of the intravenously administered fibrinogen is shown in figures 1 and 2. Also shown in figure 2 are the sedimentation rates, clotting times and clot retractions ob-

* The thrombin used was prepared by the low temperature ethanol-water method.^{3, 15} The unit given here is the same as that reported previously.³

tained with E. J. The data suggest that after intravenous infusion of fibrinogen, at least partial equilibrium is reached after two days; the half life or 50 per cent turnover rate during equilibrium was about three and a half to four days in these patients. It will also be noted that during the first two days, the injected fibrinogen disappears from the circulation at a much more rapid rate; extrapolation of the equilibrium curve to "zero time" suggests that approximately half of the administered fibrinogen left the circulation before equilibrium was established.

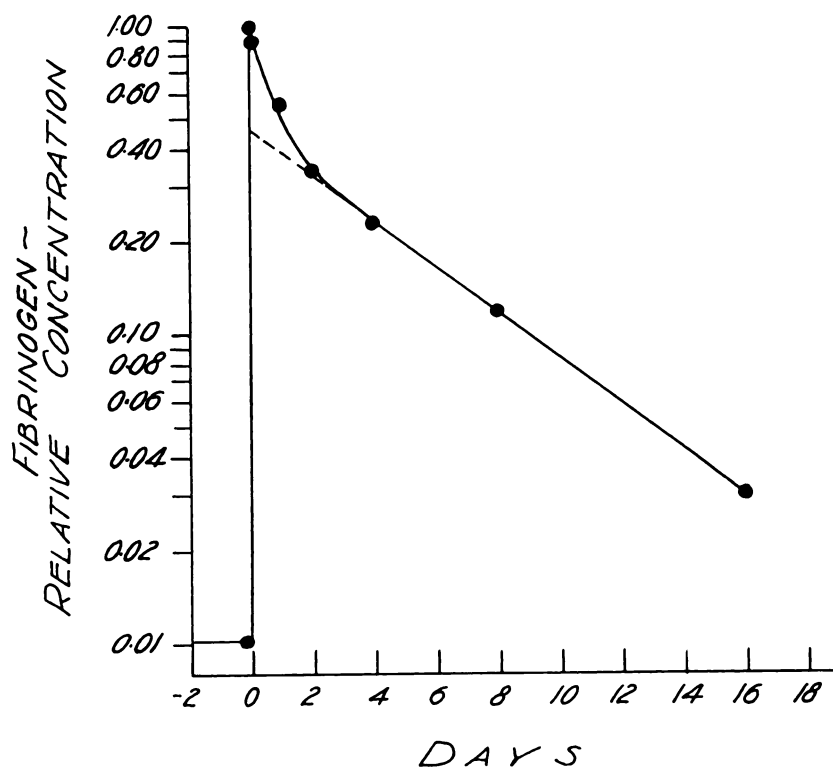


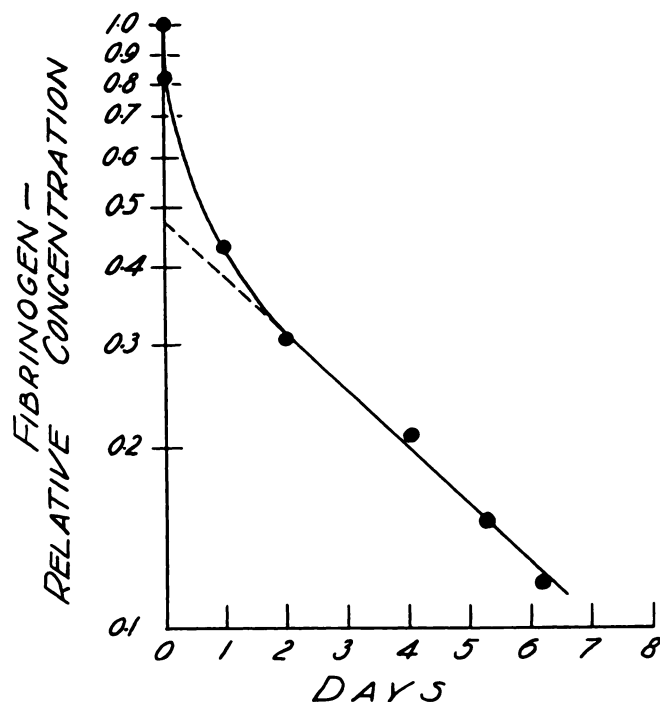
FIG. 1.—Disappearance curve of fibrinogen from plasma after intravenous administration of 1.2 Gm. fraction I to patient C. M. Relative concentration 1.00 is equivalent to approximately 100 mg. per cent fibrinogen.

The electrophoretic patterns of plasma samples from E. J. obtained immediately before the injection of fibrinogen, one hour after "zero time" and two days after "zero time" are seen in figure 3.

Muscle and skin biopsies taken prior to fibrinogen administration revealed very little fibrinogen in the connective tissues (fig. 4A and D) where it may be found normally.² As illustrated in figure 4B and E, the other plasma proteins studied in these biopsies could be found in the connective tissues as usual.² Muscle and skin biopsies taken 24 hours after fibrinogen infusion showed the presence of increased amounts of fibrinogen in the connective tissue (fig. 4C and F).

DISCUSSION

By feeding S^{35} -labelled dl-methionine or yeast and following the decreasing specific activity of fibrin, the half life or 50 per cent turnover rate of fibrinogen in three human subjects has been reported as 5.6 days.¹² Serious objections concerning the re-utilization of the radioactive label apply,¹³ and the half life of 5.6 days must be taken as a maximum value. This half life is not sufficiently different,



S.R.	05	19	25	2	1	1	mm./hr.
C.T.	∞	5	75	8	6		min.
C.R.		●	●	●	●		

FIG. 2.—Disappearance curve of fibrinogen from plasma after intravenous administration of 1.2 Gm. of ultraviolet-irradiated fibrinogen to patient E. J. Also indicated are the findings for sedimentation rate (S. R.), coagulation time (C. T.), and clot retraction (C. R.). Initial (preinfusion) relative level of fibrinogen was 0.01. Relative concentration 1.00 is equivalent to approximately 80 mg per cent fibrinogen.

therefore, from that of about four days reported here, to conclude that there is any significant increase in the rate of degradation of fibrinogen in congenital afibrinogenemia. Even if one should assume this to be an increase in degradation, with anything like normal synthesis, simple calculations would show that the fibrinogen level in the steady state would be only slightly lower than normal. With a circulating fibrinogen level of about 1 mg. per cent or less in the steady state, it is at once apparent that the fundamental defect in congenital afibrin-

ogenemia lies in an inability to synthesize fibrinogen. The exact enzymatic locus of the metabolic error must await further study.

The muscle and skin biopsies obtained prior to injection of fibrinogen revealed only traces of fibrinogen in the connective tissues. The presence of relatively large amounts of the other proteins studied indicates that the marked deficiency of fibrinogen in these tissues is not an abnormality inherent in the connective tissue but a specific deficiency in the amount of fibrinogen present. After fibrinogen was given intravenously, fibrinogen was demonstrated in the connective tissues in increased amounts; this correlates well with the findings obtained with γ -globulin in a patient with "agammaglobulinemia."²

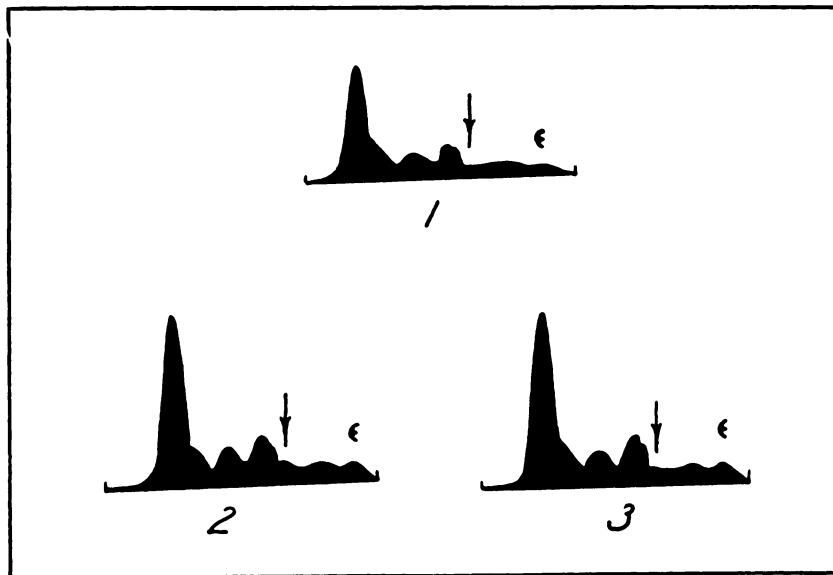


FIG. 3.—Descending electrophoretic patterns, pH 8.6, sodium diethylbarbiturate buffer $\nu/1 = 0.1$, patient E. J. 1. Before administration of fibrinogen. 2. 1 hour after fibrinogen infusion. 3. 48 hours after fibrinogen infusion. Arrows point to areas where the fibrinogen peak would normally appear.

By careful study of the disappearance of trace doses of radio-iodinated plasma albumin from the blood in normal human subjects, Sterling¹³ recently demonstrated that the total mass of exchangeable plasma albumin is approximately twice the mass of that within the blood vessels, half the total being extravascular. The presence of large amounts of plasma albumin in the cells and connective tissues of the body is in accord with this observation.² Analyzing the disappearance curves for fibrinogen as obtained in this study, it has been shown that approximately half of the administered fibrinogen disappeared extravascularly during the first two days. That part of the fibrinogen leaving the circulation appeared in the connective tissues of skin and muscle has also been demonstrated. It was found earlier that large amounts of fibrinogen are normally present extravascularly.² Similar results have been obtained for plasma γ -globulin, in a study

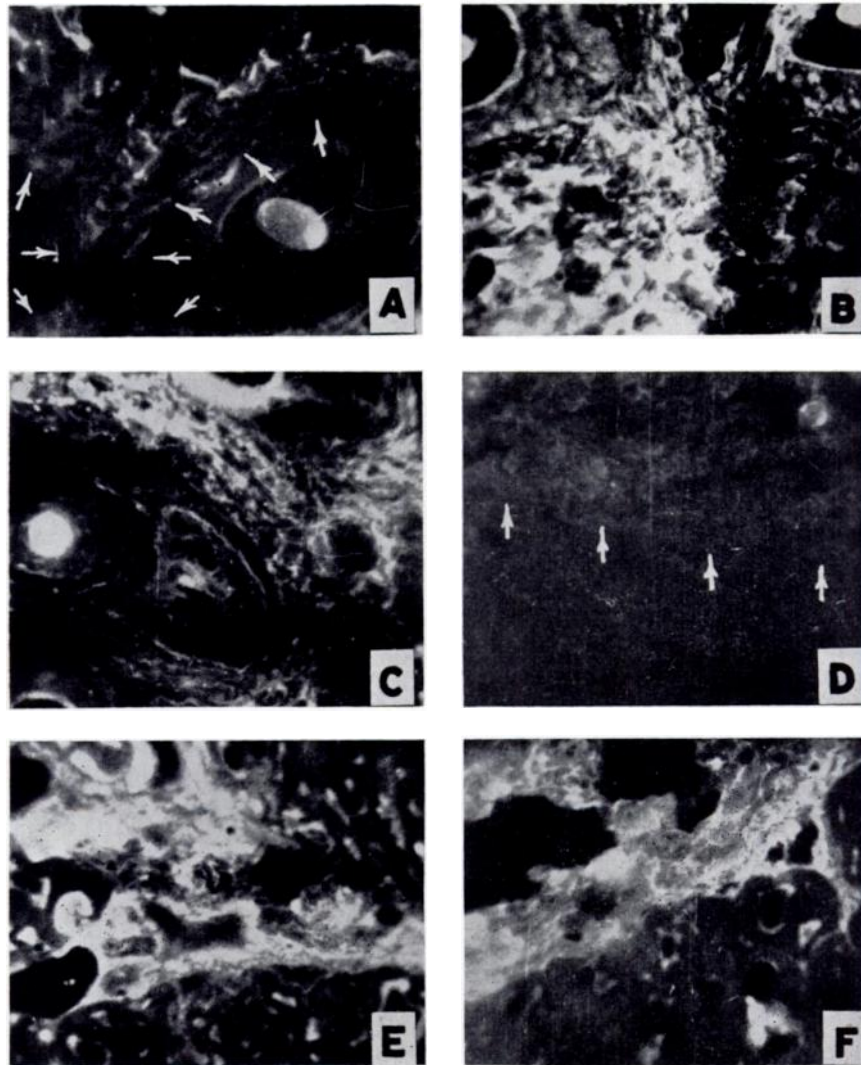


FIG. 4.— Biopsies presented were taken from patient E. J. White areas indicate fluorescence and hence sites of localization of protein being studied.

A. Section from skin biopsy taken before fibrinogen infusion, showing little fibrinogen in connective tissue (area outlined by arrows). Fluorescence of hair shaft in follicle and of elastic fibers in the area is nonspecific.

B. Section from same biopsy as figure 4A, showing large amounts of plasma albumin in connective tissue.

C. Section from skin biopsy taken 24 hours after intravenous infusion of fibrinogen showing penetration of fibrinogen into connective tissue.

D. Section from muscle biopsy showing absence of fibrinogen from connective tissue and muscle. Connective tissue above the arrows, muscle below the arrows.

E. Section from same biopsy as figure 4D, showing large amounts of plasma albumin in connective tissue.

F. Section from muscle biopsy taken 24 hours after intravenous infusion of fibrinogen showing penetration of fibrinogen into connective tissue.

of its disappearance from the serum and its appearance in extravascular sites, after injection into patients deficient in this protein.¹⁴ While it has been shown that there may be considerable differences between the distribution of one plasma protein and of another among the tissues of the body, and while the concentration of a particular plasma protein may vary from site to site or tissue to tissue,² the findings described above appear to be in accord with certain general principles:

1. The volume of distribution of the plasma proteins studied is roughly twice the plasma volume.
2. The mass of plasma protein present extravascularly is approximately equal to the mass of circulating plasma protein within the blood vessels.
3. The extravascular plasma protein is in dynamic equilibrium with the intravascular plasma protein; once equilibrium is established, a decrease in the mass of plasma protein of one "compartment" apparently results in the movement of plasma protein to that "compartment" from the other until equilibrium is again attained. The rate of synthesis of the individual plasma protein may affect the actual amount of a given plasma protein shifted before equilibrium is established.

The first two principles, of course, apply only in those situations where gross vascular disturbances such as increased capillary permeability are not present. Where increased capillary permeability is an added factor, the volume of distribution of a plasma protein and the mass of extravascular protein may become proportionately greater.

These principles assume importance in the consideration of replacement therapy with plasma proteins as well as in attempting to understand something of human physiology. The intravenous administration of fibrinogen in congenital afibrinogenemia may be used as an example: in a matter of two days the blood level attained was only half of what might have been expected on the basis of the half life of fibrinogen assuming the confinement of fibrinogen to the circulation.

SUMMARY

1. The rate of disappearance from the plasma of intravenously administered fibrinogen has been studied immunochemically in two children with congenital afibrinogenemia.
2. About half of the administered fibrinogen disappeared from the circulation in the first forty-eight hours, thus emphasizing the importance of the extravascular pool of plasma protein.
3. After the first two days, the fibrinogen followed a logarithmic decay curve, with a half life of four days; this is so close to the half life of 5.6 days estimated from radioisotope turnover studies that it indicates that the fundamental defect in these patients is a failure to synthesize adequate quantities of fibrinogen.
4. Traces of fibrinogen were detected immunochemically in the plasma of each patient; in one of them, the small amount found could not readily be attributed to prior infusion of blood or blood products.
5. The connective tissue in biopsy specimens of skin and muscle from one

patient, when examined with fluorescein-labelled antisera, was found to be specifically deficient in fibrinogen. This deficit was quickly rectified by intravenous administration of fibrinogen.

REFERENCES

- ¹ HENDERSON, J. L., DONALDSON, G. M. M., AND SCARBOROUGH, H.: Congenital afibrinogenemia. *Quart. J. Med.* 14: 101, 1945.
- ² GITLIN, D., LANDING, B. H., AND WHIPPLE, A.: The localization of homologous plasma proteins in the tissues of young human beings as demonstrated with fluorescent antibodies. *J. Exper. Med.*, 97: 163, 1953.
- ³ FERRY, J. D., AND MORRISON, P. R.: Preparation and properties of serum and plasma proteins. VIII. The conversion of human fibrinogen to fibrin under various conditions. *J. Am. Chem. Soc.* 69: 388, 1947.
- ⁴ MORRISON, P. R.: Preparation and properties of serum and plasma proteins. XV. Some factors influencing the quantitative determination of fibrinogen. *J. Am. Chem. Soc.* 69: 2723, 1947.
- ⁵ DIAMOND L. K., WOLFF, J. A., AND BORGES, W. H.: Congenital afibrinogenemia: Report of three cases. To be published.
- ⁶ COHN, E. J., STRONG, L. E., HUGHES, W. L., JR., MULFORD, D. J., ASHWORTH, J. N., MELIN, M., AND TAYLOR, H. L.: Preparation and properties of serum and plasma proteins. IV. A system for the separation into fractions of the protein and lipoprotein components of biological tissues and fluids. *J. Am. Chem. Soc.* 68: 459, 1946.
- ⁷ GITLIN, D.: Use of ultraviolet absorption spectroscopy in the quantitative precipitin reaction. *J. Immunol.* 62: 437, 1949.
- ⁸ —, LATTA, H., BATCHELOR, W. H., AND JANEWAY, C. A.: Experimental hypersensitivity in the rabbit; disappearance rates of native and labelled heterologous proteins from the serum after intravenous injection. *J. Immunol.* 66: 451, 1951.
- ⁹ POHLE, F. J., AND TAYLOR, F. H. L.: The coagulation defect in hemophilia: The effect in hemophilia of intramuscular administration of a globulin substance derived from normal human plasma. *J. Clin. Investigation* 16: 741, 1937.
- ¹⁰ WINTROBE, M. M., AND LANDSBERG, J. W.: A standardized technique for blood sedimentation test. *Am. J. M. Sc.* 139: 102, 1935.
- ¹¹ COONS, A. H., AND KAPLAN, M. H.: Localization of antigen in tissue cells. II. Improvements in a method for the detection of antigen by means of fluorescent antibody. *J. Exper. Med.* 91: 1, 1950.
- ¹² MADDEN, R. E., AND GOULD, R. G.: Turnover rate of plasma fibrinogen. *Fed. Proc.* 2: 252, 1952.
- ¹³ STERLING, K.: The turnover rate of serum albumin in man as measured by I^{131} -tagged albumin. *J. Clin. Investigation* 30: 1228, 1951.
- ¹⁴ JANEWAY, C. A., APT, L., AND GITLIN, D.: Agammaglobulinemia. A syndrome manifested by marked deficiency of circulating γ -globulin. In preparation.
- ¹⁵ ONCLEY, J. L., MELIN, M., RICHERT, D. A., CAMERON, J. W., AND GROSS, P. M., JR.: The separation of the antibodies, isoagglutinins, prothrombin, plasminogen and B_1 -lipoprotein into subfractions of human plasma. *J. Am. Chem. Soc.* 71: 541, 1949.