TABLE I. Level of Plasma Amino Acids in Rats Bearing Tumors. Averages and standard errors.

Glucose load, mg/100 g/rat/hr	Insulin, units/24 hr	Hr	No. rats	Tumor wt, g	Plasma amino acids, mg %
0	0	0	12	36.94 ± 5.46	3.97 ± .28
0	0	3	12	0.0	9.35 ± .33
0	0	3	12	2.34 ± .39	11.55 ± .45
0	0	3	8	8.98 ± 1.12	14.26 ± .88
0	0	3	10	22.63 ± .79	15.09 ± .79
0	0	3	10	37.72 ± 2.73	15.83 ± .60
0	0	6	8	0.0	15.00 ± .82
0	0	6	8	1.68 ± .27	17.91 ± 1.13
0	0	6	6	8.15 ± 1.36	20.77 ± .76
0	0	6	6	25.36 ± 1.50	20.26 ± 1.13
0	0	6	10	53.78 ± 6.21	21.64 ± 1.11
70	0	3	17	0.0	8.82 ± .09
70	0	3	17	19.1 ± 5.46	10.59 ± .25
70	16	3	17	0.0	4.48 ± .11
70	16	3	17	19.76 ± 3.41	7.30 ± .44

than in similar rats without tumors. The extent of rise shows a positive relationship to the size of the tumors at autopsy. The differences are evident in the presence and absence of insulin.

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Studies on Agammaglobulinemia VI. Hemostasis in Patients with Agammaglobulinemia.* (22202)

PAUL G. FRICK AND ROBERT A. GOOD.†

Departments of Medicine and Pediatrics, University of Minnesota, and Research Laboratories of Variety Club Heart Hospital, Minneapolis.

The discovery of agammaglobulinemia(1) has opened a new field of investigation in immunology and protein metabolism. Appreciable information concerning these patients has accumulated, especially in the field of immunology(2). In all conditions with a congenital or acquired defect of protein synthesis the problem arises whether one is dealing with an isolated error of metabolism or with a group of deficiencies. Typical examples of isolated defects of protein synthesis are the congenital disturbances of coagulation: *e.g.* afibrinogenemia, the various subgroups of hemophilia, hypoprothrombinemia, etc. Examples of combined defects of protein synthesis are found with severe liver damage, where hypoprothrombinemia is usually associated with deficiencies of prothrombin conversion factors, hypofibrinogenemia and hypoalbuminemia. The association of a number of defects can usually be traced to a dysfunction of the common organ of synthesis.

Because of the existing controversy about the site of synthesis of gamma globulins, it was considered of interest to determine the concentration of the presently known clotting factors and wherever possible to evaluate the adjustment of these factors in response to stressful situations in patients with demonstrated inability to synthesize gamma globulin. Complete tests of hemostasis were performed in 4 children with congenital agamma-

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† American Legion Memorial Heart Research Professor of Pediatrics.

globulinemia and 3 adults in whom the agammaglobulinemia was probably acquired (3). With one exception (case L.L.) all patients were males. During the course of study, one adult developed homologous serum hepatitis and expired in hepatic coma. The charges in clotting factors as the liver failure progressed will be reported below. In 2 children the fibrinogen concentration changed during a febrile episode. Otherwise all tests were carried out during a state of clinical well-being in all patients.

Material and methods. Previously described methods have been applied for the *collection and processing of blood, plasma and serum*(4), the determination of *recalcification time*(4), *thrombin time*(4), one-stage *prothrombin consumption*(4), *prothrombin concentration*(5), *labile and stable prothrombin conversion factors*(6) and *fibrinolysis*(6). *Bleeding time*—Method of Ivy(7). *Coagulation time of venous blood*—Modified 3 tube Lee-White method. *Prothrombin time*—Method of Quick(8). *Platelet count*—Method of Rees-Ecker(9). *Capillary fragility*—Rumpel-Leed test(10). *Fibrinogen determination*—Method of Ratnoff(11).

Results. The tests of hemostasis presented in Table I show no deviations from normality except for a high platelet count in all children and in one adult. Of these tests only the determination of fibrinogen, platelets and prothrombin concentration are specific and quantitative. The activity of other clotting factors can only be determined by inference from a group of additional tests. The normal consumption of prothrombin indicates that the various plasma precursors of thromboplastin (Anti-Hemophilic Globulin, Plasma Thromboplastin Component, Plasma Thromboplastin Antecedent, fourth plasma thromboplastin component of Spaet *et al.*(12) and the factor deficient in Hageman's trait(13)) must be present in normal concentration. The determination of prothrombin with the modified 2-stage method(5) revealed a normal rate of prothrombin conversion in all patients. This proves that both the labile and the stable prothrombin conversion factors are present in normal amounts in the plasma of agamma-

TABLE I. Tests of Hemostasis in 7 Patients with Agammaglobulinemia.

	Congenital group				Acquired group			Normal
	W.A., 6 yr	T.A., 1 yr	E.S., 7 yr	J.S., 7 mo	F.H., 58 yr	G.T., 29 yr	L.L., 30 yr	
Bleeding time, min.	3	2	2	2	3	1	5	max 6
Clotting time, min.	20	23	20	23	20	21	19	max 30
Prothrombin time, sec.	12.5	12.6	12.4	13.8	12.4	14.0	13.4	12.0–14.0
Recalcification time, sec.	150	90	150	120	140	150	140	max 180
Platelets, per mm³	558 × 1000	594 × 1000	390 × 1000	468 × 1000	256 × 1000	194 × 1000	189 × 1000	150–220 × 1000
Clot retraction	normal	normal	normal	normal	normal	normal	normal	
Rumpel-Leede test	neg.	neg.	neg.	neg.	neg.	neg.	neg.	
Fibrinogen, mg/100 ml	320	322	253	291	262	364	320	250–350
Prothrombin conc., U/100 ml	305	270	270	270	305	315	270	260–360
Prothrombin consumption								
Serum prothrombin time, sec. (2 hr after venipunct.)	43.4	35.7	39.0	56.0	30.6	28.4	45.6	above 25.0
Thrombin time, sec.	15.5	14.0	14.6	14.0	14.0	16.0	15.6	14.0–16.0
Fibrinolysis	absent	absent	absent	absent	absent	absent	absent	

globulinemic patients. Finally the normal thrombin time excludes any disturbance in the last phase of normal coagulation, *i.e.* in the conversion of fibrinogen to fibrin.

The changes in clotting factors in case F.H. during the course of a fatal hepatitis were in no way different from the changes observed in progressive hepatic failure in a normal individual: the drop in prothrombin concentration from 305 to 66 units/100 ml preceded the fall of fibrinogen from 262 to 110 mg/100 ml and the terminal thrombocytopenia of 72,000 per cumm. The temporary rise of fibrinogen to 449 mg/100 ml in patient J.S. during the course of a febrile episode after the administration of typhoid vaccine is also characteristic of the response observed in normals. Similarly a rise of fibrinogen from 210 to 428 mg/100 ml occurred in patient E.S. during an attack of pneumococcal pneumonia. Upon recovery the fibrinogen level fell to a normal level of 240 mg/100 ml. At no time during the course of these episodes was it possible to detect any gamma globulin in the serum of either patient by electrophoresis. It appears, therefore, that the changes in coagulation factors observed in certain disease states occur in agammaglobulinemia in identical fashion as in normal individuals.

Discussion. The elevation of the platelet count in all 4 cases of congenital agammaglobulinemia and in one adult, who probably had an acquired type of agammaglobulinemia, points toward some functional aberration in the bone marrow. It is of interest in this connection that agammaglobulinemia is characterized by other abnormalities in the hematopoietic system. Among these is the virtual absence from the lymph nodes, spleen and bone marrow, of plasma cells in all cases thus far studied(14). In certain instances absence of eosinophils from the peripheral blood and bone marrow, neutropenia and lymphopenia(3) have reflected the disturbed function of the reticuloendothelial system.

The normal concentration of the plasma clotting factors has several fundamental implications. (1) It suggests that none of the protein factors involved in the coagulation of blood is a gamma globulin. (2) It indicates that synthesis of all the coagulation moieties produced by the liver is intact in patients with agammaglobulinemia. The liver is the source of fibrinogen, plasma thromboplastin component, prothrombin and the stable and labile prothrombin conversion factors. (3) It supports the concept that the metabolic defect underlying agammaglobulinemia is an isolated deficiency of a single enzyme system. Observations thus far reported(2), including the data submitted here, indicate that synthesis of all proteins, save gamma globulin and the closely related beta 2 globulin is normal in these patients. It also seems of significance that changes in clotting factors during disease states occurred in normal fashion. All these findings are in keeping with previously published data(3) which showed no evidence of abnormality of hepatic function in patients with agammaglobulinemia. These data permit the conclusion that synthesis of gamma globulin and antibody is not directly associated with synthesis of the protein factors involved in hemostasis and support the concept that gamma globulin and antibody are not formed in the parenchymal cells of the liver.

Summary and conclusions. 1. The activity or concentration of the clotting factors recognized at the present time have been determined in 7 patients with agammaglobulinemia. Four of the patients had the congenital-hereditary form of agammaglobulinemia and three had the acquired form of the disease. 2. An elevated platelet count was observed in 5 of the 7 patients. No abnormalities in plasma clotting factors were found. 3. Data presented are consistent with the following conclusions: (a) none of the plasma coagulation factors is a gamma globulin, (b) agammaglobulinemia is probably the result of an isolated deficiency in protein synthesis, (c) the deficiency of gamma globulin synthesis in these patients does not involve the liver but rather depends on an anomaly of protein metabolism existing elsewhere in the reticuloendothelial system.

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Water Intake of Guinea Pigs as Parameter in Oral Drug Therapy of Experimental Irradiation.*† (22203)

FRIEDRICH ELLINGER.

Naval Medical Research Institute, National Naval Medical Center, Bethesda, Md.

In the study of the effects of drugs on X-ray-induced mortality of experimental animals, the use of peroral route is preferable in many instances to injection because of increased bleeding tendency exhibited by some laboratory animals. This is particularly true with respect to guinea pigs. In this species the pronounced bleeding tendency may cause fatal bleeding at the site of injection. Oral drug administration is usually performed by tube feeding, to control amount of medication as well as possible. This procedure is, however, not suitable for irradiated animals since these are particularly sensitive to infections. The small lacerations caused by such forced feeding experiments may open ports of entry for bacteria and thus superimpose a death rate caused by infection, which would becloud true results of such studies.

Administration of water-soluble drugs with the drinking water appeared, therefore, as a method of choice. Since, obviously, the amount of drug given in this manner would depend on water consumption of experimental animals, it was necessary to obtain information about their drinking habits.

Methods. Fifty-one male guinea pigs of the Institute stock with an average weight of 450 g (range 250-660 g), were kept in individual cages in air-conditioned quarters. Preliminary investigations had indicated that guinea pigs kept on our standard diet B, consisting of guinea pig pellets, lettuce, and a half orange daily, were drinking quite irregularly. To force experimental animals to drink, lettuce and oranges were withdrawn from the diet. Instead they were offered 235 cc of water to which was added 50 mg vit. C. The water was

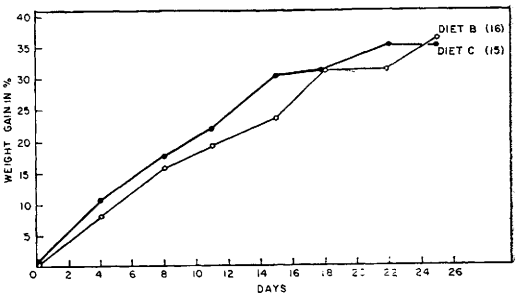

FIG. 1. Comparison between wt gains of guinea pigs kept on standard diet B and experimental diet C (dry food and drinking water plus 50 mg vit. C/235 cc). Figures in parentheses in this and subsequent curves designate No. of animals.

* The opinions or assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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