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Blood Volume Studies in Pernicious Anemia and Non-Tropical Sprue. (19926)

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It is well recognized that patients with pernicious anemia in relapse tend to retain fluids. Clinically, this may be manifested by minimal edema of skin and subcutaneous tissues or by frank dependent edema. Clinical evidence of increased water retention may be evident during the early phase of effective therapy as shown by increase of peripheral edema and occasionally by the appearance of pulmonary edema. Meulengracht(1) demonstrated that persons in relapse showed decreased diuresis following ingestion of large quantities of water. Vaughan(2) reported retention of fluid in 12 patients with pernicious anemia in relapse from the onset of treatment to the peak of reticulocytosis after which there was a period of diuresis and return to normal fluid balance. Gibson(3) reviewing the work of many investigators, noted no general agreement in reports of plasma volume changes in patients with pernicious anemia. Using Evans blue dye (T-1824) he observed a fall in plasma volume and rise in red blood cell and total blood volume after treatment. He made no studies during the period of reticulocytosis. These variable results plus the frequently observed discrepancy of a sharply elevated reticulocyte peak after specific anti-pernicious anemia therapy, with no increase in hemoglobin or red blood cell values, prompted the following investigation. Hemodilution resulting from increased plasma volume could explain this seeming anomaly.

Methods. This study included one patient with non-tropical sprue and four persons with typical Addisonian pernicious anemia in relapse. Each case in the latter group showed hyperchromic macrocytic anemia, histamine-fast achlorhydria, and megaloblastic bone marrow. Roentgen examinations of gastrointestinal tract, urinalyses and blood chemistry determinations disclosed no abnormalities. The patients were treated with either intramuscular injections of liver extract, vit. B_{12} or citrovorum factor. The subject with non-tropical sprue showed hyperchromic macrocytic anemia, histamine-fast achlorhydria, megaloblastic bone marrow and flat glucose tolerance curve. There were frequent liquid stools containing abundant fatty acid crystals. Roentgen evidence of spasm, segmentation and "puddling" of the barium column in the small intestines was reported. He was treated with citrovorum factor intravenously. The results of this study will be presented elsewhere(4). In all patients hemoglobin and red blood cell determinations were made 3 times a week, leukocyte counts once a week, and reticulocyte estimations daily. Blood volume determinations were made twice weekly using P^{32} labeled erythrocytes(5). Red cell mass was calculated from the total blood volume and hemato-

TABLE I. Hematologic Data, Red Blood Cell and Total Blood Volumes in a Patient with Non-Tropical Sprue (N.) and 4 Patients with Pernicious Anemia in Relapse Treated with Liver Extract, Citrovorum Factor and Vit. B_{12}.

Patient	R.B.C. × 10^6	Retic., %	R.B.C.V., ml	T.B.V., ml	Day of treatment
N.	1.67	.5	810	3540	1
	1.55	2.6	800	3420	4
	1.92	14.4	895	3140	7
	2.62	4.2	990	2980	11
W.	1.85	.8	805	3610	1
	1.58	1.8	655	3120	4
	1.95	16.6	860	3490	7
	2.42	5.1	1010	3430	11
C.	1.30	1.1	355	2200	1
	1.15	2.1	346	2430	4
	1.60	17.8	460	2200	7
	1.67	21.4	475	2150	9
	2.11	7.8	530	2060	12
S.	2.94	1.3	1485	3915	1
	2.95	2.1	1380	3770	6
	3.21	4.5	1490	3720	9
	3.55	3.3	1525	3575	11
	3.87	1.3	1730	4145	17
K.	1.82	.6	930	4650	1
	1.69	.4	915	4620	4
	2.16	18.6	1080	4890	8
	2.97	8.3	1170	4110	12
	3.19	2.2	1430	4770	16

R.B.C.V. = Red blood cell vol. T.B.V. = Total blood vol.

crit, correcting the latter by a factor of 0.95 (6).

Results. All of the patients showed satisfactory hematologic and clinical remissions. Reticulocyte responses were in the optimal ranges and regeneration of red blood cells took place rapidly. (Table I.) None of the subjects with pernicious anemia showed any clinical evidence of fluid retention during the period of observation. Increase in hemoglobin and red blood cells was noted at about the same time that significant reticulocytosis was observed so that the aforementioned discrepancy did not obtain in the cases observed. Blood volume changes were not significant. As a rule, the gain in red cell mass was offset by a loss of plasma volume so that total blood volume remained constant. Since none of these subjects showed any clinical evidence of edema and no lag in increase of red blood cells occurred during the period of reticulocytosis, no comment is possible as to alterations in fluid balance when either of these phenomena is observed. Further studies are indicated in such cases.

The patient with idiopathic steatorrhea had moderate pre-tibial edema before treatment. At the peak of reticulocyte response a moderate amount of subcutaneous fluid was eliminated. Plasma volume decreased more than the proportional increase of red cell mass so that total blood volume fell during this period. This decrease of blood volume despite an increase in red cell mass was more marked 5 days later, at which time subcutaneous pretibial edema was virtually gone. Whether edema observed during pretreatment period was due to cardiac dysfunction resulting from anemia of myocardium, increased capillary permeability, alterations in electrolyte balance or serum protein alterations cannot be determined from this single case. Further studies in sprue and related diseases are contemplated.

Summary and conclusions. 1. During the period of reticulocyte response, the blood volume in 4 patients with pernicious anemia remained constant, an increase in red cell mass being balanced by reduction of plasma volume. 2. One patient with idiopathic steatorrhea,

edema and macrocytic anemia showed diminution of total blood volume despite a rise in red cell mass during the period of reticulocytosis.

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Poliomyelitis. I. Propagation of the MEF1 Strain of Poliomyelitis Virus in the Suckling Hamster.* (19927)

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The work reported here was initiated originally with the thought of developing a high titered virus which could be used to produce a more practical complement-fixation antigen for the diagnosis of poliomyelitis. Since the Syrian golden hamster appears to be less prone to spontaneous virus infections than the Swiss white mouse it was chosen for the serial transmission of the MEF1 strain(1,2), Type 2 poliomyelitis virus. While our work was in progress Casals, Olitsky and Anslow(3,4) and Selzer, Sacks and van den Ende(5) adapted the MEF1 strain of poliomyelitis virus to newborn mice, but no modification in virulence of the virus for primates was reported. During the course of our work, however, it was conceived that continuous passage of the virus in young hamsters might result in the production of higher concentrations of virus than are ordinarily attained in mice and that through adaptation or fixation for the species, in the Pasteurian sense, the virus might broaden its host range and thereby lose virulence for those species ordinarily more susceptible. Could these hopes be realized without loss of antigenicity by the virus, material progress would be made in the development of a practical diagnostic antigen as well as immunizing agents in the form of inactivated or live virus vaccines. Adaptation to new hosts, especially the chick embryo, would further expand the possibilities of research and greatly simplify the preparation of diagnostic and prophylactic antigens.

The present report describes the development of a passage strain of MEF1 poliomyelitis virus carried in 7- to 10-day-old suckling hamsters and of another strain carried in 2- to 4-day-old hamsters.

Materials and methods. Virus strains. The MEF1 Lansing type (Type 2) strain of human poliomyelitis virus was obtained as a 50% glycerine-water mouse brain suspension through the kindness of Dr. Peter K. Olitsky and Dr. Jordi Casals of the Rockefeller Institute for Medical Research, New York, N. Y. The strain was passed by intracerebral inoculation for 9 passages in Swiss white mice and was thereafter carried intracerebrally in 6- to 8-week-old Syrian golden hamsters. A virus pool was prepared from the nervous tissues harvested from the 30th to the 34th hamster passages in the following manner: the brain and spinal cord tissues were aseptically removed from paralyzed hamsters and were thoroughly ground in a TenBroeck grinder; a 10% tissue suspension of the

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† Sodium chloride 9 g; potassium chloride .3 g; calcium chloride .2 g; sodium carbonate .2 g per liter of solution.