brinogen(1), it is probable that our findings are relevant to the platelet as well as to the leukocyte.

Summary and conclusions. Fibrinogen and other plasma proteins are apparently adsorbed on the hydrophilic surface of normal leukocytes, thereby complicating the interpretation of leuko-agglutinin tests. Normal leukocytes separated from whole blood (but not leukocytes separated from defibrinated blood) were rapidly and non-specifically agglutinated when suspended in normal untreated serum. Polymerization of adsorbed fibrinogen appeared to be the mechanism of such non-specific agglutination, which could be prevented by adequate decalcification of the suspending serum. Addition of EDTA to all test sera has permitted construction of a satisfactory leuko-agglutinin test using white cells separated from whole blood.

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Effect of D-Sorbitol on Absorption of Vitamin B_{12} by Pernicious Anemia Patients.* (24314)

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It has been previously demonstrated that sorbitol in solution with Vit. B_{12} will increase oral absorption of Vit. B_{12} in non-pernicious anemia subjects as measured by an increase in serum levels. Increased absorption of Vit. B_{12} by means of sorbitol has been reported to occur in experiments with young, healthy adults(1), and intact rats(2), while limited experiments with pernicious anemia patients were inconclusive(3). Since pernicious anemia is the primary state in which increased Vit. B_{12} absorption is desired, and since it was speculated that sorbitol may have an intrinsic factor-like action, experiments were undertaken on the effect of sorbitol on absorption of orally administered Vit. B_{12} in pernicious anemia patients in remission.

Method. Each pernicious anemia patient was fed an oral dose of 2 μg of Vit. $B_{12}Co^{58}$ containing 0.25 μc of radioactivity, first alone, then together with a potent intrinsic factor concentrate, then together with crystalline D-sorbitol and finally together with both intrinsic factor concentrate and D-sorbitol. In each of these 4 tests the medication was mixed and diluted to 100 ml, and following ingestion the container was rinsed with tap water and the rinsings fed to the subject. Immediately and 24 hours after each oral dose, 1000 μg of unlabeled Vit. B_{12} was given intramuscularly. The 4 excretion studies on each patient were repeated with a second series of studies using 30 μg oral doses of $B_{12}Co^{58}$ (containing 0.5 μc of radioactivity). In all instances except where noted the amount of sorbitol used was 10 g. Radioactivity of each 24-hour urine specimen was determined with a special beaker of one liter capacity adapted to fit over and around a NaI well type scintillation crystal(4) or by counting 200 ml in

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TABLE I. Effect of Sorbitol on Absorption of Vitamin B_{12} in Pernicious Anemia Patients. (2 μg oral dose.)

Pt.	B_{12}	B_{12} + sorbitol*	B_{12} + IF	B_{12} + IF & sorbitol*
	Excretion in urine (48 hr), μg			
K.M.	.02	.02	.36	.29
A.S.	.01	.01	.17	.23
R.U.	.01	.01	.35	.31
F.W.	.02	.02 .02 (1 g) .02 (.1 g)	.14	.11
H.W.	.01	.01	.11	.17
Avg	.02	.02	.23	.22

* Dose of sorbitol was 10 g, otherwise given in parentheses.

TABLE II. Effect of Sorbitol on Absorption of Vitamin B_{12} in Pernicious Anemia Patients. (30 μg oral dose.)

Pt.	B_{12}	B_{12} + sorbitol*	B_{12} + IF	B_{12} + IF & sorbitol*
	Excretion in urine (48 hr), μg			
K.M.	.24	.09	.18	.18
A.S.	.44	.14 (21 g)	.45	.23 (21 g)
R.U.	.48	.03	.60	.48
F.W.	.16	.20	.24	.42
H.W.	.12	.12	.27	.36
Avg	.29	.12	.35	.33

* Dose of sorbitol was 10 g, otherwise given in parentheses.

a highly efficient plastic scintillator slightly larger than that previously described(5). The intrinsic factor concentrates used in these studies were either #186-1 (50 mg) previously studied by Baker and Mollin(6), Callender and Evans(7), Williams *et al.*(8) and Ellenbogen *et al.*(9) or preparation #1-R (30 mg). Each dose was administered to the patients by one individual and radioactivity of all the urine specimens of all the patients was determined by one individual.

Results. The results of the urinary excretion tests using both 2 μg and 30 μg oral doses of B_{12} are presented in Tables I and II respectively. Each of the 5 patients excreted 0.02 μg or less when given 2 μg $B_{12}Co^{58}$ alone indicating that the patients lacked ability to absorb Vit. B_{12}. None of the patients showed an increase in excretion of Vit. B_{12} when 10 g of sorbitol was given together with 2 μg of Vit. B_{12}. In one patient (F.W.) 1.0 and 0.1 g of sorbitol was also given to investigate the possibility that the 10 g dose might be too large and result in inhibition. The lower doses of sorbitol also did not cause an increase in absorption of Vit. B_{12}. On the other hand, when intrinsic factor concentrate was fed together with B_{12}, absorption of Vit. B_{12} was increased as indicated by increase in its urinary excretion. Excretion of B_{12} with intrinsic factor concentrate was 5 to 18 times that obtained with 2 μg alone or with 2 μg and sorbitol. When Vit. B_{12} was given simultaneously with intrinsic factor concentrate and sorbitol no increase in absorption was observed above what was obtained with Vit. B_{12} plus intrinsic factor concentrate. In 2 of the patients a slight increase of questionable significance was observed. The overall average excretion of the 5 patients receiving Vit. B_{12} plus intrinsic factor concentrate and sorbitol was 0.22 μg as compared to 0.23 μg obtained with Vit. B_{12} and intrinsic factor.

In the tests with the 30 μg oral dose of Vit. B_{12}, sorbitol showed no effect in increasing absorption obtained with 30 μg alone. On the contrary, sorbitol caused a marked inhibition of absorption in 3 of the 5 patients. Simultaneous administration of sorbitol and intrinsic factor concentrate together with Vit. B_{12} resulted in an average excretion of 0.33 μg as compared to 0.35 μg with intrinsic factor concentrate and Vit. B_{12} alone, indicating that sorbitol did not appear to enhance the effect of intrinsic factor with large oral doses of Vit. B_{12} in pernicious anemia patients.

These studies clearly indicated that sorbitol did not enhance absorption of Vit. B_{12} in pernicious anemia patients. Intrinsic factor is the only substance known to increase absorption of physiological amounts of Vit. B_{12} in pernicious anemia patients.

Summary. 1) In the urinary excretion test 5 pernicious anemia patients excreted radioactivity corresponding to an average of 0.02 μg of Vit. B_{12} when fed 2 μg of Vit. B_{12} orally and excreted an average of 0.02 μg when fed 2 μg of Vit. B_{12} together with 0.1 to 10 g of sorbitol. These patients excreted an average of 0.23 μg when fed 2 μg of Vit. B_{12} with intrinsic factor and an average of 0.22 μg when 2 μg of Vit. B_{12} were fed together with intrin-

sic factor and 10 g of sorbitol. 2) These 5 anemia patients excreted an average of 0.29 μg when fed 30 μg of Vit. B_{12} and 0.12 μg when fed 30 μg of Vit. B_{12} together with 10 to 21 g of sorbitol. The same 5 patients excreted an average of 0.35 μg when fed 30 μg of Vit. B_{12} with intrinsic factor, and excreted 0.33 μg when 10 to 21 g of sorbitol was fed together with 30 μg of Vit. B_{12} and intrinsic factor. 3) Sorbitol had no effect in promoting absorption of Vit. B_{12} in pernicious anemia patients.

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Clinical and Laboratory Studies of Mumps II. Detection and Duration of Excretion of Virus in Urine. (24315)

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The cultivation of mumps virus in tissue culture was first reported by Weller and Enders(1). Tissue cultures were prepared by suspension of amniotic membranes from embryonated hen's eggs in a manner similar to that previously described by Maitland and Maitland(2). Subsequently Kilham and Murphy(3) propagated mumps virus in mouse embryo tissue culture employing a method developed by Earle and associates. Henle and others(4) using mono-layer cell culture demonstrated cytopathic effects produced by mumps virus. Henle and Deinhardt(5) later described the use of the same method in the primary isolation of virus. We(6) reported studies on cytopathic effects of virus in tissue culture and described methods for use by the diagnostic virology laboratory in isolation of virus from a variety of patient specimens. The presence of virus in urine specimens was demonstrated in 6 patients. In the preparation of these specimens it was found necessary to dilute urine 10-fold in saline to avoid a non-specific "toxic" effect on the tissue culture cells. We felt, however, that this dilution could reduce the chances of detecting virus in lightly infected urine specimens. We here describe an improved method for preparation of urine for viral isolation and report on duration of excretion of virus in 13 additional patients.

Patients. A study was made of 11 male adults, 1 male child and 1 female child, each of whom had an illness clinically characteristic of mumps. With the exception of the 2 children, all patients were hospitalized at the Clinical Center of the Nat. Inst. of Health or at the Nat. Naval Medical Center, Bethesda, Md. Clinical diagnosis was made on physical examination at the time the patient was placed on the study. Date of onset of parotid or submaxillary gland swelling or tenderness was recorded. Follow-up examinations were made of the salivary glands at the time subsequent specimens were collected from hospitalized patients. Days of illness were numbered in relation to the first day on which the patient observed salivary gland swelling or pain. In