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

TABLE I. Results of Urine Culture for Mumps Virus.

Patient	Day of illness (after salivary gland swelling or pain) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
R.W.				+					+		+									
R.C.				+		0		0			0									
G.D.		+			+		+					+		+		0				
W.K.				+			+		+					0		0		0		
D.M.				+				0			0		0							
R.S.			0		+					0		0		0			0			
G.S.				0	0			0						+						
C.M.		0		0		+		0		0			0		0		0		0	
J.G.			+		+			+		+		+			0					
H.O.				+			+		+		0					0				
R.O.						+					0		0					0		
D.K.			+		+			+		0		0				0			0	
P.P.	+																			
Total (+)	1	1	2	5	4	2	3	2	3	1	1	2	0	2	0	0	0	0	0	29
Total tested	1	2	3	7	5	3	3	6	3	4	5	4	3	4	2	4	2	2	2	65
% (+)	100	50	66	71	80	66	100	33	100	25	20	50	0	50	0	0	0	0	0	45

0 = tested, and negative. Blank = not tested.

none of these patients were other prodromal symptoms noted earlier than 24 hours prior to salivary gland involvement. Clinically detectable orchitis was not present in any of the patients.

Methods. The first voided morning urine specimen was collected in a sterile container. Saliva specimens collected concurrently were handled as previously described(6). Within 6 hours of collection, urine specimens were prepared by 2 methods for inoculation. *Preparation by dilution*: 0.2 ml of the urine specimen was diluted 10-fold in Hanks' balanced salt solution. *Preparation by concentration*: Approximately 15 ml of urine was clarified by centrifugation at 4°C for 10 minutes at 1500 rpm. 11.4 ml of the supernatant urine was then centrifuged at 4°C for 90 minutes at 40,000 rpm in a Spinco Ultracentrifuge, Model L. The pellet from this was then resuspended in 1.14 ml of Hanks' balanced salt solution. 0.1 ml amounts of both preparations were inoculated into HeLa and primary monkey kidney roller tube cultures. Mumps virus was identified in tissue cultures exhibiting characteristic cytopathic effects by hemagglutination inhibition tests using standard mumps antiserum(6).

Results. A total of 65 urine specimens from 13 patients were collected during the period following onset of parotid swelling. Virus was isolated from the urine as early as the first day of swelling and as late as the fourteenth day. The overall experience in isolation of virus as correlated with day of illness is shown in Table I. Forty-five percent of all specimens tested within 19 days after onset yielded virus. During the first 14 days of illness 55% of the specimens were positive and during the first 9 days of illness 70% of the specimens were positive. Virus was recovered from each of these 13 patients. Fifty-seven of the 65 specimens were prepared by both dilution and concentration methods. 25 of the 57 yielded virus: in 8 both preparations, and in 17 the concentration preparation only, were positive. In the remaining 8 of 65 specimens studied, only the dilution preparation was made: of these, 4 were positive.

The relative efficiency of monkey kidney and HeLa cell tissue culture in primary isolation of mumps virus from urine was compared. 27 of 29 isolations were made in monkey kidney cells only and the remaining 2 isolations were made in both monkey kidney and HeLa cultures. In no instance was virus isolated in HeLa cultures alone.

In 7 of 13 patients virus was detected in the urine after clinically evident parotitis had subsided whereas in 5 patients, urine specimens were negative after swelling had disappeared. Urine specimens from the 13th patient were not tested after the first day of illness.

On 63 occasions saliva specimens were collected when urines were obtained. A large number of the saliva specimens, however, were contaminated by a fungus and tissue culture studies for virus on these specimens were unsatisfactory. On 13 occasions, however, virus was detected in urine specimens but not in satisfactory saliva specimens collected at the same time. In this and the previous study (6) virus has not been recovered from saliva or swabs of the oral cavity at a time later than the 8th day of illness. Virus has been recovered from the urine of 8 patients at times later than this in these studies.

Discussion. The success of concentration of infected tissue culture fluids by high speed centrifugation for demonstration of hemagglutinin(6) suggested the use of this technic for preparation of urine specimens prior to inoculation. The superiority of this technic over the previously used method of preparation seems evident from the data presented here. This concentration procedure results in a 100-fold greater inoculum of the urine specimen that is presumably virus. Monkey kidney cell cultures seem preferable to HeLa cells for detection of mumps virus. This confirms previous work(6) but is not at odds with another observation that mumps virus once adapted to tissue culture propagates well and produces a cytopathic effect in HeLa cells. The concentration technic would seem to be adaptable to studies of other viruses and its use in isolation of another virus is being reported(7). Use of the concentration preparation and monkey kidney cell cultures inoculation results in a relatively efficient method for isolation of mumps virus during the early stages of illness. It is possible that blind serial passage of the negative tissue cultures (unpublished) and use of the hemadsorption test(8) may increase the percentage of urines that are positive.

It is of interest that virus was isolated from urine as early as the first day of illness and at a time less than 20 hours after the appearance of the first symptom. This is consistent with the concept that at the onset of parotid swelling widespread dissemination of virus has occurred. Furthermore, excretion of virus seems to continue during the phase of acute clinical illness. The mechanism by which virus is excreted in urine has been speculated upon elsewhere(7). The role that virus present in urine plays in the clinical illness is under investigation. In addition, viruria persisted in some patients for a period of time after all symptoms or signs of illness had disappeared. The small number of patients in this study and relatively short periods of observation (less than 20 days) did not make it possible to determine whether some mumps patients might excrete the virus for much longer periods. The possibility of this occurrence has epidemiologic implications as to where virus may be harbored between epidemics.

Summary. 1. Specimens of urine from mumps patients were concentrated by ultracentrifugation prior to tissue culture inoculation. This concentration resulted in 3 times as many mumps virus isolations as simultaneously tested portions of the same urine prepared by simple dilution. 2. All isolations were made in primary monkey kidney cell cultures; only 2 were made also in simultaneously inoculated HeLa cultures and none were made in HeLa cultures only. 3. Mumps virus was recovered from the urine of each of 13 patients within the first 14 days after onset of salivary gland pain or swelling. Virus was isolated from urine as early as the first and as late as the 14th day of illness. No testing was done beyond the 20th day.

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