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## Mumps III. Comparison of Methods for Detection of Viruria. (27666)

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In rhesus monkey kidney cell cultures inoculated with specimens containing mumps virus a distinctive cytopathic effect (CPE) appears(1), and the infected tissue culture cells adsorb erythrocytes (hemadsorption–HAD)(2). The following investigation was undertaken to compare these newer technics with the older methods of hemagglutination (HAG) and embryonated hen's egg inoculation in detection of virus in urine.

*Methods. Patients.* Patients with suspected mumps were studied while hospitalized at the Clinical Center Nat. Inst. of Health, at the Nat. Naval Med. Center in Bethesda, or in a few instances while at home. Days of illness were numbered in relation to the first day the patient observed parotid or submandibular gland swelling or pain.

*Specimens.* Since virus can be detected in urine for a considerably longer period than in oral or throat swabs(3) urine specimens were studied. The first voided morning specimen was collected in a sterile container, stored at 4°C and delivered to the laboratory within 2 to 5 hours. In a few instances when the specimen was inadvertently left at room temperature, a later morning urine was collected and both specimens were studied. Urines were collected every Monday, Wednesday and Friday while the patient was under observation.

Specimens were prepared by ultracentrifugation as previously described(3) and the pellet was suspended in medium 199 with 0.5% gelatin. The prepared specimens were immediately inoculated into cell cultures and embryonated hen's eggs.

Blood was collected from most patients on the first and last day of observation.

*Cell cultures.* Rhesus monkey kidney cell cultures were used within 7 days of receipt in the laboratory. They were inoculated with 0.1 ml of the prepared specimen and observed daily thereafter. Fluids were passaged at least once at the time when CPE was judged maximal or otherwise 13-14 days after inoculation.

*Hemadsorption.* At the time of passage or discard the supernatant fluids were removed and 0.3 ml of a 0.4% guinea pig or human group O erythrocyte suspension in saline was added to the cell culture tubes. After standing for 20 minutes at 4°C, the tubes were observed microscopically for hemadsorption. Controls of uninoculated monkey kidney cell cultures were included.

*Hemagglutination.* Serial 2-fold dilutions of the cell culture supernatant fluids were prepared in 0.5-ml amounts of 0.9% saline solution. An equal volume of a 0.4% suspension of guinea pig erythrocytes was added and the tubes shaken. After the tubes had remained standing at room temperature for 90 minutes the highest dilution showing hemagglutination was determined.

*Hemagglutination-inhibition.* Control and anti-mumps guinea pig or rabbit sera(1) were

treated with an equal volume of undiluted *Vibrio cholerae* filtrate and allowed to stand overnight at 4°C. The sera were then placed in a 56°C water bath for 30 minutes. Serial 2-fold dilutions of the serum were prepared in 0.25-ml amounts in 0.9% saline solution. To each dilution was added an equal volume of positive supernatant fluid diluted to contain 4 hemagglutinating units. The tubes were shaken and placed in a 37°C water bath for 1 hour. At the end of the incubation period, 0.5 ml of a 0.4% guinea pig erythrocyte suspension was added to each tube and the tubes read after remaining 90 minutes at room temperature. The highest dilution of serum inhibiting hemagglutination was determined and the supernatant was considered to contain mumps virus if a difference of at least 4-fold existed between control and antimumps sera.

*Embryonated hen's eggs*(4). Each of six 5-6-day-old embryonated hen's eggs was inoculated by the amniotic route with 0.1 ml of the prepared urine specimen. The embryos were incubated an additional 7 days at 37°C. Amniotic fluids were removed and fluid from each embryo was tested for hemagglutinin with human group O and guinea pig erythrocyte suspensions at 4°C and at room temperature. Negative amniotic fluids were pooled and passaged an additional 3 times.

*Complement fixation (CF).* Acute and convalescent sera obtained from patients were used in the complement fixation test as described by Bengston(5) using commercially available mumps (viral) antigen. A 4-fold or greater increase in titer was considered evidence of infection.

*Identification of virus.* The final proof of presence of virus in a particular urine was based on a positive hemagglutination-inhibition test of cell culture or egg embryo fluids. When supernatant fluids from cell cultures exhibiting CPE or HAD failed to hemagglutinate guinea pig erythrocyte suspensions, fluids from 10 cell cultures were pooled, concentrated as previously described(3), and tested for presence of hemagglutinin.

*Results.* Thirty patients suspected of having mumps or of being in the incubation phase were studied. Four patients had no confirmatory laboratory evidence of mumps and 5 patients had only an antibody rise in paired sera. These 9 are not considered further. In the remaining 21 patients, all had a virus isolation and each of the 17 so tested also had a CF antibody rise. The 110 urines collected from these 21 patients form the basis of this report.

Fifty-eight of the 110 urine specimens were positive for mumps virus.

A direct comparison of the cell culture and hen's egg methods of virus isolation is shown in Table I. Cell culture method failed to detect virus in only 1 of the 2 specimens positive in eggs, whereas the latter method did not yield virus in 44 of 45 urines positive in cell culture.

Table II shows a comparison of the results of CPE and HAD methods in primary cell culture of the urine specimens. The HAD test was positive in only 18 of 37 (49%) CPE positive urines but was useful in indicating virus presence in 7 of 7 CPE negative or questionable cultures. With subsequent passage in cell cultures an additional 20 urine specimens became CPE positive and 26 specimens became HAD positive. In places of apparent disagreement between the 2 methods of detection of virus in primary culture, subsequent passage was similarly helpful (Table II).

TABLE I. Cell Culture and Hen's Egg Methods of Detecting Mumps Virus in 110 Tested Urines.

| | Eggs+ | Eggs− | Eggs NT* | Total |
|---|---|---|---|---|
| Cell culture + | 1 | 44 | 12 | 57 |
| Cell culture − | 1 | 46 | 6 | 53 |
| Totals | 2 | 90 | 18 | 110 |

* NT = not tested.

TABLE II. CPE and HAD Methods of Detecting Mumps Virus in Primary Cell Cultures of 110 Urines.

| | HAD+ | HAD− | NT | Total |
|---|---|---|---|---|
| CPE + | 18 | 15* | 4† | 37 |
| CPE − | 3‡ | 42 | 6 | 51 |
| CPE ? | 4§ | 16 | 2 | 22 |
| Total | 25 | 73 | 12 | 110 |

* 10 HAD + on passage.
† 4 HAD + " "
‡ 3 CPE + " "
§ 4 CPE + " "

TABLE III. HAD and HAG Methods of Detecting Mumps Virus in Cell Cultures of 110 Urines.

| | HAG+ | HAG– | NT | Total |
|---|---|---|---|---|
| HAD + | 45 | 6 | 0 | 51 |
| HAD – | 0 | 13 | 44 | 57 |
| HAD NT | 0 | 0 | 2 | 2 |
| Total | 45 | 19 | 46 | 110 |

Of the 51 HAD positive urines 45 (88%) were HAG positive. HAG was not detected in 13 HAD negative specimens (Table III).

Urine specimens collected in the earlier days of illness were more likely to yield mumps virus than later specimens. The percentages of positive urines were: days 1-5, 80%; 6-10, 69%; 11 and thereafter, 23%. These figures are similar to percentages in an earlier study(1).

Urines from 2 patients were positive later in the illness (day 15) than have been reported previously. Subsequent urines were negative in both of these patients. One patient with a positive urine on day 15 had a biphasic illness with parotitis early and orchitis later.

On 7 occasions when the first voided urine had been left at room temperature and a later fresh specimen was collected, virus was present either in the first specimen only or in both specimens, and never in the later specimen only.

A summary of experience with the 58 positive urines is shown in Table IV.

*Discussion.* For isolation of mumps virus from urine, monkey kidney cell cultures are overwhelmingly more sensitive than the embryonated hen's egg. The latter method also was more time consuming, and the cost of raw materials 50% greater.

In the primary cell culture studies CPE was a more sensitive indicator of presence of virus than HAD. It is possible that if the cell cultures had been studied earlier HAD might have been present before CPE. At the time of a positive test for HAD, HAG was usually also present and the former was only a slightly more sensitive indicator of virus. Despite being less sensitive than the CPE and only slightly more sensitive than the HAG, HAD is a simple and rapid procedure for detection of virus. It is important to note that HAD and HAG testing was done without prior knowledge of CPE readings.

TABLE IV. Summary of Experience with Virus Detection in 58 Mumps Positive Urines.

| | |
|---|---|
| CPE + | 57 |
| HAD + | 51 |
| HAG + | 45 |
| Hen's egg + | 2 |

Concentration by ultracentrifugation was not done routinely on supernates from CPE, HAD, and HAG negative cell cultures and it is possible that some of these could have been shown virus-positive after such treatment. However, past experience as well as the negative results of concentration of 20 such supernates not heretofore listed in this study are against this possibility.

The first voided specimen of urine even when inadvertently left at room temperature for a number of hours seems preferable to a fresher but later and less concentrated specimen.

Although virus was found in the urine as late as the 15th day of illness, there is no evidence from our studies to indicate mumps virus excretion for the prolonged periods observed in patients infected with salivary gland virus(6).

Of the 5 patients with CF antibody rise but no virus isolations from urine, 2 were studied only late in their illness and virus may have been excreted earlier. An additional explanation for this observation might be that the illness which resembled mumps clinically was due to an antigenically similar virus(7).

*Summary and conclusions.* Of 110 urine specimens collected from 21 patients with culturally confirmed mumps, 58 were positive for mumps virus. In 56 virus was recovered by cell culture only, in one by hen's egg method only, and one urine by both methods. In the primary cell cultures cytopathic effect was the most sensitive indicator of virus. Hemadsorption was demonstrated on passage, however, in 90% of specimens exhibiting a cytopathic effect. Hemagglutinin was present in 88% of fluids from cultures with cells positive for hemadsorption. Mumps virus was detected as late as the 15th day of illness and in 80% of urines collected from these patients during the first 5 days of illness.

It is concluded that cell cultures are superior to embryonated hen's eggs in detecting viruria, that in the cell culture system cytopathic effect is a more sensitive indicator of virus than hemagglutination or hemadsorption, but that the latter is a simple and rapid confirmatory technic.

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## Amprolium 10. Influence of Egg Yolk Thiamine Concentration on Chick Embryo Mortality. (27667)

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A thiamine deficiency can be produced in adult chickens and eggs(1,2,3) by feeding sufficiently high dietary levels of the coccidiostat amprolium* [1-(4-amino-2-*n*-propyl-5-pyrimidinylmethyl) - 2 - picolinium chloride · hydrochloride]. The drug is deposited in egg yolks at concentrations related to its level in the diet(2) but is innocuous to developing embryos or newly hatched chicks over a wide range of diet levels studied(2,3). At very high levels of amprolium in feed, embryo mortality was observed and correlated with depressed free thiamine concentrations in the egg(3).

From the many experiments(1,2,3) used to evaluate effects of amprolium on reproduction of adult chickens, data were obtained on yolk thiamine concentrations, and hatch results associated with these yolk levels. These and other data in this report made it possible for the first time to estimate minimum free thiamine concentration in yolk for optimum development of White Leghorn chick embryos.

*Methods and procedures.* Adult White Leghorn female chickens were housed in single cages of 3-deck batteries in a constant temperature room at 72°F or on wire-floored pens in a wood frame house. They were fed *ad libitum* for 21 days a commercial breeder ration containing amprolium and then nonmedicated diet for 12 days. Daily collections of eggs from the hens were set weekly in an incubator at 99½°F and 85% humidity. Pooled samples of yolk from 3 eggs were obtained from each day's collection and analyzed for free thiamine and amprolium. In other studies, weekly collections were employed(1,2,3). Fertile eggs were obtained from battery-reared hens by use of artificial insemination, and from hens in wire-floored pens by use of 1 male to each 10 females. Hatchability was calculated from the number of normal chicks obtained from fertile eggs, and this proportionality was plotted after arc sin transformation, according to Snedecor(4).

*Results.* Free thiamine concentrations in yolks averaged 2.91 ppm during the first 3 days that .2% amprolium was fed to hens. Thereafter, its concentration declined to a low of .38 ppm by day 11 on medication (Fig. 1). On the other hand, the hatch showed no significant change ($P<.05$) until day 8, when it declined from a control of 85 ± 2% (mean ± standard error) to 65, and thereafter continued to decline reaching its lowest level by day 21 (Fig. 1). Preliminary (unpublished)

* Coccidiostat from Merck & Co., Inc., Rahway, N. J.