mals. The activity of barbarin in animals which might feed vigorously on the wild plant is unknown. It is unlikely, however, that enough barbarin would be transmitted to cow's milk to be a potential hazard in the human consumption of this milk(10).

Summary. The antithyroid potency of barbarin, a phenylthiooxazolidone obtained from various species of *Barbarea* and *Reseda luteola,* was assayed by its inhibition of radioiodine uptake in rat and man. In both species it was found to be approximately 50% as active as goitrin (vinylthiooxazolidone).

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Cytopathogenicity of Mumps Virus in Cultures of Chick Embryo and Human Amnion Cells.* (26761)

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Primary monolayer cultures of chick embryo (CE) and human amnion (HA) cells have been utilized in assay of a number of animal viruses. To our knowledge, cytopathogenicity of mumps virus for these cells has not been previously reported. In the present paper cytopathic changes are described which were induced in these systems by mumps virus.

Methods and materials. Cell cultures. Methods of preparation and maintenance of primary cultures of HA and CE cells have been described(1,2). HA cells were maintained on Enders' medium(1) and CE cells on a medium consisting of 2.5% heat inactivated calf serum, 1.5% chick embryo extract, 48% bovine amniotic fluid (BAF), and 48% Hanks' Balanced Salt Solution (BSS). For plaque assay 3% Difco agar in BSS was added to an equal volume of a solution of 10% inactivated calf serum and 90% BAF. Neutral red dye (final concentration 1:20,000 or 1:40,000) was added 5-7 days after inoculation of virus. Dilutions of virus were incubated with cells for two hours at 37°C prior to overlay with agar. *Virus.* Two strains of mumps virus were employed: a) Enders strain, passaged 47 times in the amniotic cavity and 6 times in the allantoic cavity of the embryonated egg; b) a strain which caused CPE in HA cells on primary isolation,‡ and was subsequently passed 8 times in these cells. *Serological tests.* Complement fixation, neutralization and hemagglutination inhibition tests with anti-mumps guinea pig serum§ and hemadsorption of guinea pig erythrocytes were performed by

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‡ This was one of several strains of mumps virus isolated in HA cells by Dr. S. Kibrick in this laboratory.

§ Obtained from Microbiological Associates, Inc., Washington, D.C.

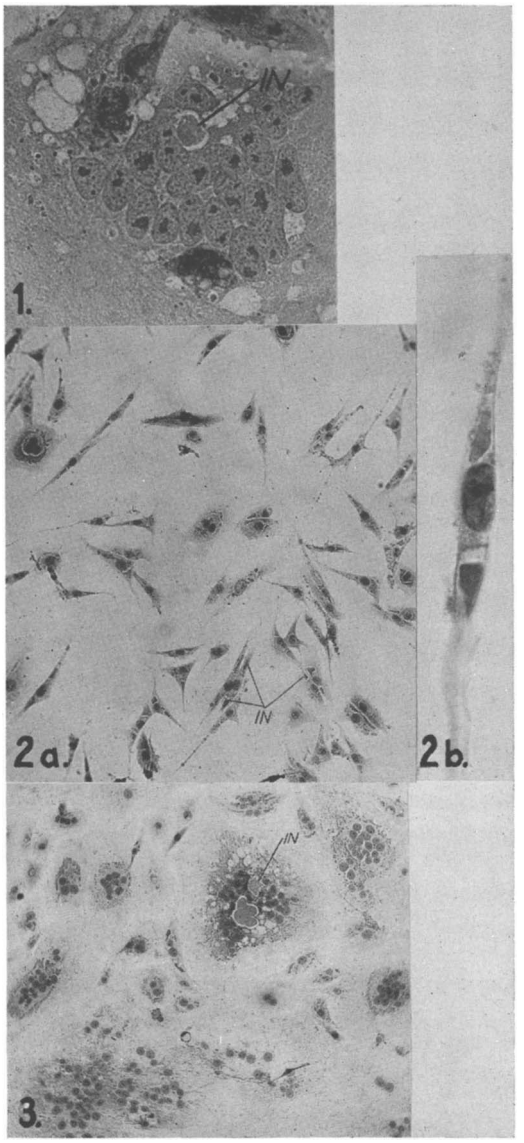

FIG. 1. Chick embryo cells 6 days after inoculation of mumps virus, demonstrating a giant cell with eosinophilic cytoplasmic viral inclusion body (IN). Mag. × 350. Hematoxylin and Eosin stain.

FIG. 2, a and b. Human amnion cells 8 days after inoculation of mumps virus demonstrating "spindle" cells with eosinophilic cytoplasmic inclusion bodies (IN). Mag. × 250 and 750. Hematoxylin and Eosin stain.

FIG. 3. Human amnion cells 6 days after inoculation of mumps virus; demonstrating giant cells with eosinophilic cytoplasmic inclusion bodies (IN). Mag. × 190. Hematoxylin and Eosin stain.

standard methods(3,4).

Experimental. Chick embryo cells. Cytopathogenic effect (CPE) induced by the Enders strain in CE cells was characterized by formation of multinucleated giant cells (Fig. 1), increased cellular refractility, and eventually almost total destruction of the cells. Cytopathic changes were observed as early as 4-5 days after inoculation of larger quantities of virus and by the 10th to 12th day with minimal reacting doses (10^{-6} TCD_{50}/ml). Eosinophilic cytoplasmic inclusion bodies surrounded by haloes were apparent after fixation and staining. Similar CPE was obtained upon serial passage of fluids from infected cultures to fresh preparations of chick cells. Cytopathic effects were inhibited by addition of mumps antiserum. Concomitantly with cell destruction, complement fixing antigen and hemagglutinin for guinea pig, chicken and frog erythrocytes in low concentrations (1:8-1:32 dilutions of culture fluid) were demonstrated.

With agar overlay, viral plaques (1-2 mm in diameter) were observed 6-8 days after inoculation of virus, but the toxicity of the neutral red dye for chick embryo cells made this technic less reliable than infectivity assay by tube titration.

In CE cultures infected with the Enders strain hemadsorption of guinea pig erythrocytes was observed 12-24 hours before cytopathic changes appeared.

In marked contrast to the behavior of the chick adapted strain in CE cells the human amnion cell strain failed to induce CPE or adsorption of erythrocytes.

Human amnion cells. Infectivity titers of the human amnion cell strain in HA cells ranged between $10^{-5.5}$-$10^{-6.3}TCID_{50}$/ml. CPE was noted as early as the third day after inoculation and the end point was attained after 10 days. Two types of CPE were observed. The first was characterized by formation of spindle-shaped cells (Fig. 2a,b) and the second type by appearance of multinucleated giant cells (Fig. 3). Both effects were observed in the same cultures, though often one or the other predominated. Attempts by terminal dilution technics to isolate a clone inducing only spindle or giant cell formation were unsuccessful.

Viral plaques (1-3 mm in diameter) were observed in HA cell cultures under agar 5

days after inoculation of virus. Plaque margins were not sharply defined and there was a tendency for plaques to enlarge eccentrically, creating a comet-like effect. A satisfactory correlation was noted in comparative titrations by the plaque and end point dilution technics.

In HA cells infected with the amnion cell adapted virus, hemadsorption of guinea pig erythrocytes preceded appearance of cytopathic changes by 12-24 hours. Neither of these manifestations of viral activity were observed following addition of the Enders strain to HA cell cultures.

Discussion. Multiplication of mumps virus in cultures of chick embryo tissues and membranes has been reported by several investigators(5,6,7,8,9,10). None, however, described cellular destruction associated with viral increase with the exception of Taylor (9), who observed inhibition of cell migration from fragments of infected chick amniotic membrane. Taylor interpreted his findings as evidence of viral cytopathogenicity—an interpretation that has been questioned by other workers(7,11). The present experiments show that in monolayer cultures of chick embryo cells chick adapted mumps virus consistently produces extensive cellular degeneration.

One can visualize several conditions that might account for the failure of previous investigators to observe CPE in fragments of infected chick embryonic membranes which were employed in all these earlier studies. For example, the insusceptibility of this tissue in contrast to that of the embryo itself may depend upon innate resistance of the individual cellular components. But it is also to be considered that resistance of the membranal cells is determined by preservation of their natural arrangement and inter-relationships under the conditions of cultivation employed. When cells are dispersed by trypsin and propagated in monolayers, as in the cultures we have studied, such "community" or histological resistance may be lost and the susceptibility of individual cells may then be readily demonstrable. Some support for this hypothesis is, perhaps, afforded by unpublished experiments of one of us (JFE), in which no evidence of viral multiplication was obtained in suspended fragment cultures of human amniotic membrane inoculated with poliovirus Type I. In human amnion cell monolayers this agent, of course, multiplies readily and causes complete destruction of the cells.

Although not directly relevant to the observed discrepancies with chick adapted virus in chick tissue cultures it is apparent from our results and those of others that cytopathogenicity for a given cell system may also be determined by the adaptive status of the virus. In our hands infection of HA cells with the human amnion adapted strain was followed by extensive cytopathic changes terminating in cell destruction whereas chick cells proved entirely refractory to this agent, as were HA cells to the chick adapted virus. Takemoto and Lerner(12) likewise failed to demonstrate CPE in cultures of HA cells after addition of a chick-adapted mumps virus. Henle and Deinhardt(13,14), demonstrated the capacity of freshly isolated strains to multiply and induce CPE in human cell lines and monkey kidney cells in contrast to chick adapted strains which could not be serially propagated in these cells. Recently Brandt(15) found that of 4 strains of mumps virus that had been cultivated in chick embryos only 2 that had received the least number of passages in this host were capable of continuous multiplication in mammalian cell cultures.

The viruses of mumps and measles are at present considered to be quite unrelated. It is of interest, therefore, that the cytopathic changes induced by mumps virus closely resembled those produced by measles(1). Thus both giant cell and spindle cell formation were regularly seen in mumps infected HA cells along with intracytoplasmic inclusion bodies. The latter were first described by Brandt(10). Intranuclear inclusions so characteristic of measles virus infection, were, however, not observed.

Summary. Cytopathogenicity of 2 strains of mumps virus for primary cultures of chick embryo (CE) and human amnion (HA) cells was studied. The chick adapted Enders strain induced formation of multinucleated

giant cells and extensive cellular destruction in CE cells but did not visibly affect HA cells. Multinucleated giant and spindle-like cells were observed in cultures of HA cells infected with a low passage human amnion adapted strain, which did not visibly affect CE cells. With agar overlay technic viral plaques were obtained with both strains in susceptible cell monolayers.

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Toxic Effects of Potato Sprouts and of Solanine Fed to Pregnant Rats. (26762)

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During one phase of a long-time feeding study involving reproduction in rats(1), some of the animals were fed a potato diet containing an estimated 7% of sprouts (wet wt.). On this diet, several of the rats delivered dead pups or pups that died within 2 or 3 days. The adults showed no toxic symptoms except, possibly, a lactation deficiency, and successfully raised subsequent litters after the sprouts had been eliminated from the diet.

The present report describes studies undertaken to confirm the toxic effects of sprouts and to isolate and identify the toxic factor involved. Exploratory tests confirmed the toxicity of the sprouts, both in a synthetic basal diet and in ground lab chow, when they were fed at a 10% level. When a sample of sprouts was dried at 50°, ground, and extracted successively with petroleum ether and methanol, the toxic factor was found to have been concentrated in the methanol extract. The methanol extract was found to be rich in alkaloids (Mayer's test). Furthermore during preliminary attempts at fractionation of the components of the methanol extract followed by biological tests, it was noted that those fractions which were exposed to mineral acid treatment lost an appreciable proportion of their toxicity. The latter observations led to the hypothesis that a glycosidic alkaloid such as solanine might be the toxic factor involved.

The general toxicity of sprouted potatoes and of solanine is well documented(2-5). However the literature appears to contain no studies of the effects of sprouted potatoes or of solanine on reproduction of animals. Consequently, potato sprouts in the frozen state were acquired for further sprout feeding tests and for isolation of solanine for inclusion in the biological tests. Preliminary tests indicated that the aglycone solanidine was inactive, and the latter alkaloid was therefore not included in the studies reported below.

Isolation of solanine. Frozen potato