as described above, could be responsible. It is of interest that these 2 strains, both isolated from children, are highly encephalitogenic when inoculated into rabbit cornea.

Underwood *et al.* (4, 5) observed the emergence of IUDR-resistant HSV during treatment of experimental keratitis in rabbits. After 6 or 7 days of treatment with 0.1% IUDR, new lesions appeared and 3 of 4 isolates from such lesions were resistant to IUDR *in vitro*. In the rabbit HSV keratitis model employed in our laboratory, treatment was administered for only 5 days, and we failed to induce IUDR-resistance in any of the HSV isolates obtained 1 and 2 days after the end of IUDR treatment. Again we are at a loss to explain the differences in findings.

From the results presented here we conclude that the use of IUDR in a large majority of patients with HSV keratitis has had little impact to date on the emergence of IUDR-resistant HSV strains. The rabbit model suggests that IUDR-resistance may not be a frequent outcome of short IUDR treatment (5–7 days). While IUDR-resistant HSV can be produced by laboratory manipulations, the dynamics of virus populations may not favor the emergence of IUDR-resistant strains in human disease. The problem should be reinvestigated after several additional years of IUDR use.

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Correlation of Infectivity and Hemagglutinins of Reoviruses* (33420)

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Reovirus type 3 usually produces much less hemagglutinins (HA) than types 1 or 2 (1), whereas the infectivity titer of all three types reaches about the same level. Lerner *et al.* (2) suggested that HA units and infectivity units are correlated, since both were inactivated at about the same rate when the reoviruses were incubated over a prolonged period at 37°. Zalan and Labzoffsky (3) on the other hand showed that the HA of type 3 reached maximal titer only after a 2-week incubation time at 37° whereas maximal infectivity titer was reached 4–5 days after inoculation. They also reported that after incubation at 37° for 3–4 months the infectivity declined and disappeared, but the HA titer remained constant for as long as 8 months; hence there was no parallel increase or decrease of HA and infectivity. Also Usmankhodzhayev and Zakstelskaya (4) reported that the stability of infectivity and HA of these viruses was not the same at 37°. The infectivity decreased steadily over a period of 40 days, whereas the HA of reovirus types 1 and 2 remained constant, or showed a slight increase over the first 30 days and then declined rapidly; reovirus type 3 HA however was rapidly inactivated. From previous investigations we also demonstrated an infectious and noninfectious HA

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moiety of reoviruses. The proportion of infectivity and HA units in these two moieties differed (5). The following investigations were carried out to clarify the correlation of infectivity and HA of reoviruses, using prolonged incubation at 37° and density gradient separation of infectious and noninfectious HA moiety.

Materials and Methods. Virus growth. The three reovirus prototypes (type 1, Lang; type 2, Jones; type 3, Abney) were propagated in primary monkey kidney tissue culture cells (MKTC) grown in roller tubes. The MKTC were maintained in high cystine altered Eagle's medium (6) without serum. The tubes were inoculated with about 10^5 TCD_{50} (multiplicity of 1) and left in roller drums at 37° for various periods. After 4–5 days cytopathic effects (CPE) could be observed with all virus types and after 7–9 days the destruction of the cell sheet was complete. A sample of tubes was periodically harvested by repeated freezing and thawing and the HA and infectivity titer was determined.

HA and infectivity titration. HA titers were determined by the micro technique described by Sever (7) using 0.85% saline buffered with 0.01 *M* phosphate at pH 7.4 as diluent, and human type O RBC. Infectivity titers were determined by inoculating MKTC tubes with serial 10-fold dilutions of virus and determining the 50% end point. The final examination of the tubes was on the seventh to ninth days after inoculation.

Density gradient centrifugation. As previously described (5) the virus harvests were mixed with CsCl (0.46 g/cm^3) and centrifuged in an SW-39 head of the Spinco model L centrifuge for 16–20 hr at 35,000 rpm. Ten to 12 fractions were collected and assayed for HA and infectivity.

Results and Discussion. The MKTC tubes infected with each reovirus type were maintained in roller drums and harvested periodically. The HA and infectivity titer of these harvests were determined; results are shown in Fig. 1. The infectivity titer of all three virus types started to decline from the fifth day on. After 28 days, for example, less than 1% of the virus remained infectious. The HA titers, however, remained virtually constant

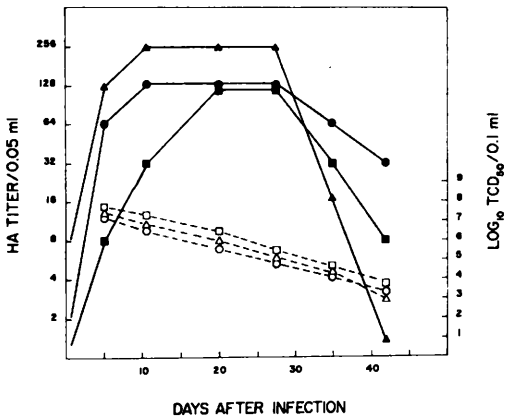

FIG. 1. Hemagglutinin and infectivity titers of reovirus types 1, 2, and 3 after prolonged incubation at 37°. Monkey kidney tissue culture tubes were inoculated with each virus type, respectively, and harvested after various time intervals. Solid symbols represent HA titers; open symbols, infectivity titers (TCD_{50}/0.1 ml): (○), reovirus type 1; (△), reovirus type 2; (□), reovirus type 3.

for types 1 and 2 and increased for type 3 over a period of 28 to 30 days, when the HA titers also started to decline. It was interesting to note that the HA of reovirus type 2 was inactivated more rapidly than the HA of types 1 and 3; moreover type 3 HA developed at a slower rate than types 1 or 2 HA.

Since the ratio of infectious units and HA units changed during the period of observation, harvests of all three reoviruses were analyzed periodically in CsCl gradients to determine if the persisting or increased HA activity was due to complete virions (density 1.38 g/cm^3) or to the unifectious HA moiety (density 1.30 g/cm^3). The results are shown in Fig. 2. At each harvest period the two typical HA moieties were separable, the denser one coinciding with the infectivity. However the changes, i.e., increases or decreases of HA activity were equally reflected in both moieties. Therefore it could be concluded that the temporal increase of HA activity of type 3 or persistent HA titer of types 1 and 2 with concurrent loss of infectivity were not due to a preferential assembly of uninfectious HA particles (presumably lacking RNA), or fragmentation of virions. The infectivity residing in the virus RNA seemed to be rather

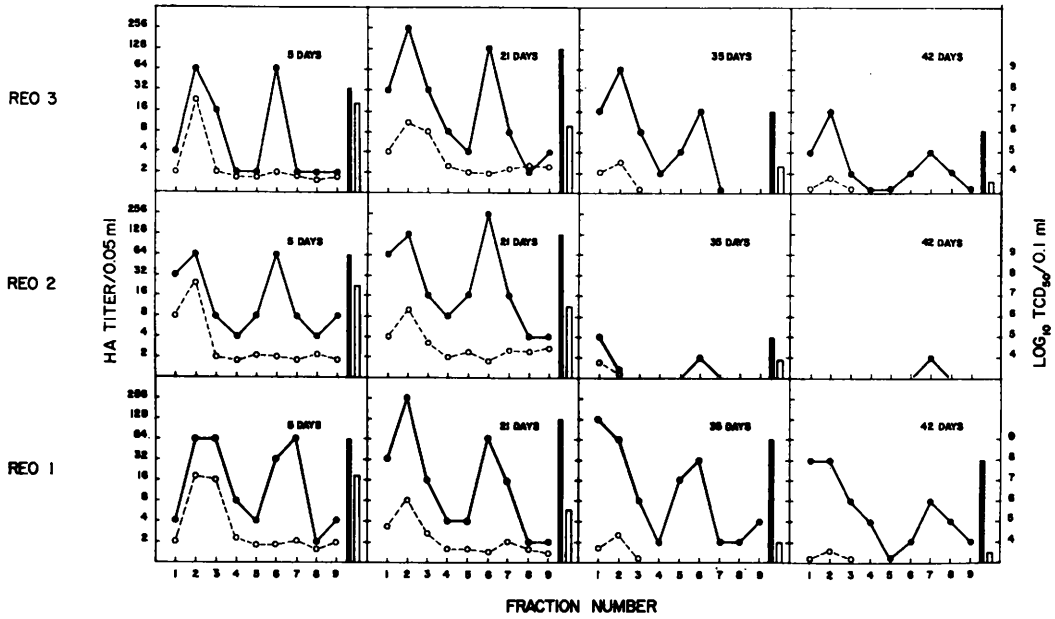

FIG. 2. Separation of infectious and noninfectious HA moieties by CsCl density gradient centrifugation of reovirus types 1, 2, and 3 after prolonged incubation at 37°: (●) HA titer of fractions; (○) infectivity titer of fractions. Fraction 1 represents the bottom (most dense) fraction of the gradient. The two HA moieties corresponded in density with the previously established density of 1.38 g/cm³ (infectivity) and 1.30 g/cm³ (top component) (5). The solid bars = HA titers, the open bars = infectivity titers of the tissue culture fluids before centrifugation.

stable at elevated temperatures but the virion may have lost its ability to attach to susceptible cells (8). Therefore it may be postulated that incubation at 37° makes the viral coat unfit for the infection process but more suitable for hemagglutination. This, together with a further dispersion of virus particles from cell fragments may account for the loss of infectivity and rising, or at least constant, HA titer of reoviruses.

In comparing our results with previous work we partially agree with results reported by Zalan and Labzoffsky (3) who also found that incubation at 37° increased the HA titer of reovirus type 3, we however could not confirm their finding that the infectivity remained unchanged over a period of 2 months; here our results confirm the results reported by Lerner *et al.* (2); but on the other hand, these authors found that infectivity and HA were inactivated about at the same rate at 37°. To some degree our results also agree with the report by Usmankhodzhayev and Zakstelskaya especially with regard to reovirus types 1 and 2; however these authors did not find an increase of reovirus type 3 HA after a prolonged incubation at 37° (type 3, Dearing was used in their study).

It is difficult to pinpoint the reasons for the partial agreement and disagreement of these reports but the type of cell cultures and media, stationary tissue cultures versus rolling cultures, or virus strains which were used may be the reason. However most of the results indicate that it is impossible to strictly relate the reovirus infectivity to its potential to agglutinate red blood cells, since even virions isolated by density gradient centrifugation were losing their infectivity but maintaining their HA or as seen with type 3 even increasing their HA, after prolonged incubation at 37°.

Summary. All three reovirus prototypes were grown in MKTC; the infected cultures were maintained at 37° for up to 42 days. During this time period infectivity and HA of the reoviruses were periodically deter-

mined. The infectivity of all three types steadily declined to less than 1% of the maximal titer after 30 days. The HA of types 1 and 2 remained unchanged over a period of 28 days; type 3 HA increased over this time interval and titers of 128 HA units were reached. The infectious and noninfectious HA moiety of the periodic harvests were separated in a CsCl gradient. The changes (type 3) or stability of HA (types 1 and 2) were equally reflected in both moieties; no accumulation of noninfectious HA was found which would account for the persistence of HA and concurrent loss of infectivity.

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Intestinal Phosphatase and Fat Absorption* (33421)

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Alkaline phosphatase of intestinal origin has been identified by electrophoresis in the serum of some subjects and not in others (1–4). This component has a slower mobility in starch gel at pH 8.6 than the liver and bone fractions which constitute the remainder of the activity of normal serum. Geneticists have called attention to this polymorphism by claiming an association of blood groups O and B and the ABH secretor status in saliva with the presence of this particular isoenzyme of alkaline phosphatase in the serum (1–5). Most of these studies were carried out on serum collected without regard for meals. Recent work by Langman *et al.* (6) indicates that this relationship is important. They found that certain subjects lacking the intestinal component in the fasting state will develop it several hours after ingesting a meal with a high fat content. They reported that this occurred only in subjects who secreted blood group substances in their saliva; regularly in those of blood groups O but B only occasionally in those of blood group A. In a previous report by Warnock (7), it was found that the intestinal component could be produced in subjects lacking it in the fasting state. Blood type and secretor status were not reported in that study. Therefore the present study was carried out on subjects who were of blood type A or who were nonsecretors. Again, in every case, the intestinal component could be detected electrophoretically in the serum 3 and 5 hr after ingestion of 56 g of butter fat as cream. These studies indicate that although quantitative differences between secretors and non-secretors may be found, these differences are not qualitative. This distinction is important as a basis for understanding the significance of the rise in the serum and its relationship to fat absorption.

Material and Methods. Fourteen ambulatory medical students, physicians, and hospital personnel, aged 20–35, were selected on the

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