the inoculum and was correlated with the severity of the disease as measured by LD_{50}. The time at which the decrease in water consumption began did not appear to be correlated with the inoculum LD_{50} within a range of 1.5 $\log_{10}$ virus concentrations. Thus, the appearance of disease symptoms with a virus concentration which caused 15% mortality and 33% reduction in water consumption occurred at the same time as with a virus concentration which caused 96% mortality and 78% reduction in water consumption.

On the assumption that water consumption reduction is a valid criterion for disease onset, the curative action of amantadine HCl appears to be adequately demonstrated by the trials of this study. This is reflected by the decrease in mortality, increase in survival time and increase in apparently healthy animals in influenza A2/Bethesda/10/63, as well as in an increased proportion of healthy animals among the survivors of influenza A2/AA/2/60 mouse infections.

Summary. The first disease symptom of influenza A2-infected mice was a reduction in water consumption. The degree of reduction with influenza A2/Bethesda/10/63 was virus-dose related whereas the time of appearance of this symptom was not. Amantadine HCl treatment of mice infected with influenza A2/Bethesda/10/63 and with influenza A2/AA/2/60 beginning 12 hr after the appearance of this disease symptom resulted in an increase in the percentage of survivors, an increase in survival time, an increase in the number of healthy animals, and an increase in the proportion of healthy to diseased animals of those which survived.

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Identification of Human Hepatic Glucokinase and Some Properties of the Enzyme* (33396)

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Glucokinase, a hexokinase type enzyme with a high K_m toward glucose, has been established as an important factor in glucose phosphorylation by rat liver (1–4). It was at first reported that glucokinase did not occur in human liver (5); subsequently, it was detected there by starch gel electrophoresis (6). The present study deals with the definite identification of a glucokinase in human liver and with some of the properties of the enzyme.

Methods. Liver tissue was obtained from a 24-year-old Negro male during gastrointestinal surgery for vagotomy and pyloroplasty. He had been fasted overnight but was otherwise well-nourished and had no signs of liver disease; he received intravenous glucose during operation. A 1-g wedge of liver was removed after hemostatic sutures had been placed but not tightened.

The tissue was placed in ice immediately after excision and homogenized within 5 min

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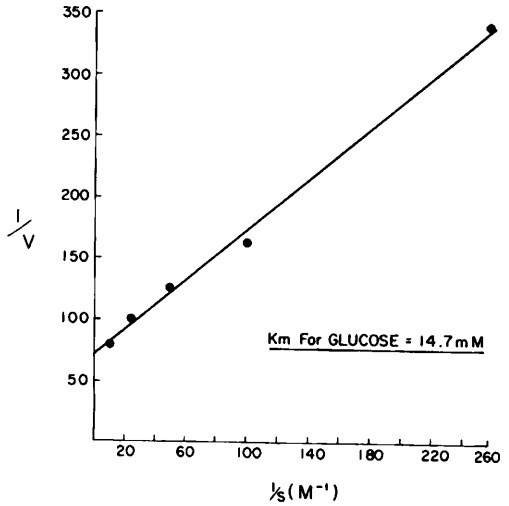

FIG. 1. Determination of the K_m for glucose of human hepatic glucokinase: human glucokinase was separated from attendent low K_m hexokinases by passage of the 105,000*g* supernate through Sephadex G-100. The pooled glucokinase activity was concentrated by ultrafiltration and used for the K_m determination. Concentrations were as follows: ATP (5 m*M*), $NADP^+$ (0.5 m*M*), Mg^{2+} (7.5 m*M*), Tris (100 m*M*), glucose-6-phosphate dehydrogenase (0.4 unit), and glucose (4, 10, 20, 40, and 100 m*M*). The enzyme was added last and the rate followed at 340 mμ and at 29°. The initial rate was taken as the linear portion of the curve after the initial 3 min, during which time the steady state was reached. Velocity of the enzymatic reaction is expressed as ΔOD_{340}/min.

in a medium consisting of 0.02 *M* Tris, 0.005 *M* EDTA, 0.004 *M* $MgSO_4$, 0.15 *M* KCl, 0.010 *M* β-mercaptoethanol, pH 7.4. A 1:1 homogenate was prepared and spun at 105,000*g* in the Spinco model L centrifuge for 60 min, and the ATP-D-glucose phosphotransferase (hexokinase) activity for the supernate was assayed at 0.5 m*M* and 100 m*M* glucose by the spectrophotometric method of Vinuela *et al.* (2). Other techniques were applied as before (7, 8): vertical starch electrophoresis (9); histochemical staining for hexokinase (10); molecular weight estimations on calibrated Sephadex G-100 columns (8); antibody production to rat hepatic glucokinase (11).

Results. In general, mammalian liver contains at least one hexokinase with a high K_m (10^{-2} *M*) toward glucose (glucokinase) and a variable number of hexokinases with low K_m values toward glucose (10^{-4} *M* or less) (11, 13). The glucokinase content of the 105,000*g* supernate from human liver was 8.5 milliunits/mg of crude protein; the value for hexokinases with a low K_m toward glucose was 5.5. These correspond to about 0.6 and 0.4 units/g of liver, respectively, as compared to 1.5 and 0.4 for rat liver. The value for glucokinase, which is obtained as the difference between the hexokinase activity at 100 m*M* glucose and that at 0.5 m*M* glucose, is probably low because a substantial proportion of the low K_m hexokinase activity of human liver has been found in the present study to be inhibited at 100 m*M* glucose. In any case, the glucokinase activity in human liver is at least 1.5-fold that for low K_m hexokinases, as compared to 4-fold in rat liver. The K_m toward glucose of human glucokinase is about 14.7 m*M* (Fig. 1) as compared to the 10–20 m*M* reported for the rat liver enzyme (12).

Four hexokinases are observed in a starch gel electrophoretic pattern for the 105,000*g* supernate from human liver (Fig. 2). The slowest migrating form corresponds to rat type I, the next fastest to rat type II (faint in both human and rat liver), and the next to rat type III. These three bands all arise from low K_m hexokinases, as do those of the rat. The fourth band is glucokinase, since it stains only at high glucose concentration, and corresponds almost exactly in migration rate to the rat glucokinase.

The low K_m hexokinases can be separated from glucokinase as shown in Fig. 3. Upon spectrophotometric assay, the low K_m fraction is inhibited by 100 m*M* glucose to the extent of 27% as compared to the control run at 0.5 m*M* glucose. From electrophoretic evidence, it is the type III which is inhibited; rat type III is also inhibited by high glucose concentrations (13).

One distinguishing characteristic of glucokinases from monkey, rat, turtle, and frog is an apparent molecular weight by the Sephadex G-100 gel filtration method of 48,000; about half the value of 91,000 for the low K_m hexokinases from the same species

(11). Similar findings were obtained for the human liver hexokinases (Fig. 3).

Glucokinases from various mammals are inhibited by an antibody prepared against 1200-fold purified rat hepatic glucokinase, while the low K_m hexokinases are not (11). In a like manner human glucokinase is inhibited 85% by the antibody to rat glucokinase whereas the low K_m hexokinases of human liver are not.

Two low K_m hexokinase bands are found by starch electrophoresis of extracts of human adipose tissue (Fig. 2), confirming the report of Brown *et al.* (6). The specific activity of the hexokinase in these extracts of human subcutaneous adipose tissue was 3.1 milliunits/mg of protein. Estimations, by the

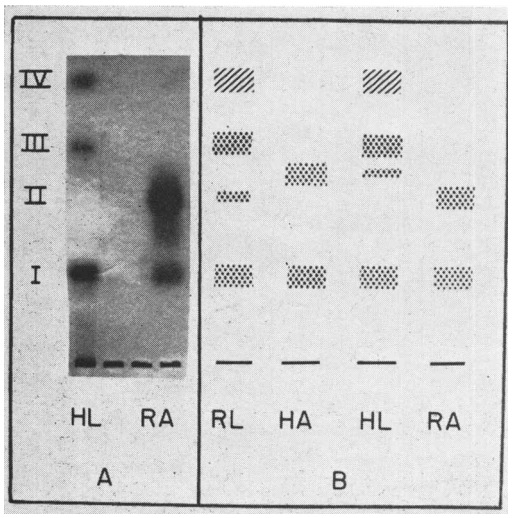

FIG. 2. Starch gel electrophoresis of human hexokinases: A. Photograph of electrophoretic pattern of human liver and rat adipose tissue supernates. The direction of migration is upward, toward the anode. The type IV band in human liver appears only at high glucose concentrations indicating that it is a high K_m hexokinase. The type III band stains more intensely at 0.5 m*M* glucose than at 100 m*M* glucose (not shown), indicating that it is inhibited by high glucose concentrations. B. Schematic representation of electrophoretic patterns for human and rat hexokinases from liver and adipose tissue. Cross hatched bands appear only at high glucose concentration. Stippled bands stain at both gluocse concentrations. None of these bands appeared if ATP was omitted from the staining mixture. HL, human liver; HA, human adipose tissue; RL, rat liver; RA, rat adipose tissue.

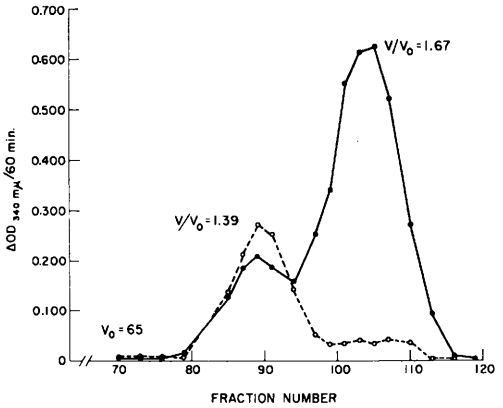

FIG. 3. Sephadex G-100 gel filtration of human hepatic glucokinase and hexokinase: Human liver supernate (1 ml) was applied to a Sephadex G-100 column (1.5 × 92 cm) equilibrated with 100 m*M* Tris, 5 m*M* EDTA, 10 m*M* β-mercaptoethanol, and 4 m*M* $MgSO_4$, pH = 7.0. (—), total hexokinase activity at 100 m*M* glucose. It should be noted that the low K_m activity, eluted with V/V_o of 1.39, is inhibited by high glucose concentrations. This column was calibrated with proteins of known molecular weight (8) and yielded an apparent molecular weight of 48,000 for glucokinase (V/V_o = 1.67). (- - -), low K_m hexokinase activity.

Sephadex G-100 gel filtration method, of the apparent molecular weights of these adipose hexokinases yield a value of 91,000, the same as for the low K_m hexokinases of human liver and rat adipose tissue. The rat and human adipose enzymes are not inhibited by an antibody to rat liver glucokinase.

Summary. Glucokinase activity has been estimated in human liver. The human enzyme is similar to that of the rat in K_m for glucose, electrophoretic mobility, apparent molecular weight, and reactivity to an anti-glucokinase prepared against the rat enzyme.

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The Early Detection of Picodnavirus ×14 by Immunofluorescence* (33397)

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There are now a large number of small DNA-containing animal viruses which by analogy with the RNA-containing picornaviruses have been called "picodnaviruses" (1). The known physical and chemical properties of members of this group have been tabulated recently (2). To date, only rodent picodnaviruses are known to be capable of autonomous replication in susceptible cells, while the human and simian members appear to be unable to multiply unless host cells are also infected with adenovirus (3–5). The ×14 is a rodent picodnavirus serologically related to the H_3 hamster osteolytic virus (6). The ×14 exhibits a $T = 3$ pattern of icosahedral symmetry similar to that of K rat virus (7, 8). Because of its similarity to the defective picodnaviruses (adeno-associated satellite viruses), ×14 may serve as a useful model of an autonomous small DNA virus. However, to date it has been impossible to detect ×14 virus with certainty until 3 or 4 days after inoculation of susceptible host cells. The use of fluorescein labeled antibodies to follow the growth cycle of satellite virus in the presence of its helper (9) led us to apply similar methods in the present experiments to the possible early detection of ×14 virus.

Materials and Methods. A strain of ×14 virus kindly supplied by Dr. F. E. Payne was grown in primary tissue cultures prepared from Sprague-Dawley rat embryos as described previously (7). Confluent monolayers on coverslips in Leighton tubes were inoculated at a multiplicity of approximately 1 $TCID_{50}$/cell. The cells were harvested at various times after infection, frozen and thawed three times and assayed for hemagglutinating activity (HA) and infectivity. Companion coverslip cultures were fixed in acetone for 10 min and stained by the direct fluorescent antibody technique as described previously (9). The hemagglutination pattern test was carried out in a plastic plate using 0.4% guinea pig erythrocytes (10). For convenience, the plates were held at 4° overnight before reading although equally high titers were obtained when the test was carried out at room temperature. The HA titers were expressed as the reciprocal of the highest

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