The possibility that improved muscular function was due to the additive effect of cellular entry of plasma glucose plus fructose cannot be ruled out.

The results suggest that: a) Fructose is removed from plasma at a rapid rate since the levels remained constant during exercise and the removal equalled the input rate of 0.90 g/min. b) Threshold level of the sarcolemmal membrane for entry of fructose is much lower than that for glucose since at an extracellular fluid level of fructose near 10 mg % muscular work could be performed. Plasma glucose had to be increased to nearly twice the fasting level in order to similarly enhance work. c) Fructose traverses the muscle cell membrane at a sufficiently rapid rate to contribute to muscular work. d) Fructose can be effectively utilized by skeletal muscle as a major source of energy under some circumstances.

Summary. 1. Observations were made upon the effect of infused fructose on work capacity of a young male subject with an unusual metabolic deficiency of striated muscle in which there was a failure to form lactic acid under anaerobic conditions. The defect was tentatively ascribed to inability to utilize glycogen and was manifested clinically by limited exercise tolerance. 2. Duration of work was greatly improved by infusion of glucose or fructose. Fructose was effective at much lower plasma concentration than glucose. Therefore, fructose can traverse the muscle cell membrane and contribute significantly toward the fuel for muscular work in this patient.

ADDENDUM: The defect in this case has recently been demonstrated to be an absence of phosphorylase a and b in muscle.

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Some Biological Characteristics of Asian Influenza Isolates.*† (24740)

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Occurrence of an antigenic variant of influenza A in Southeast Asia which rapidly spread producing pandemic influenza throughout the world in 1957 has been described(1). Detailed studies in many laboratories have shown that closely related strains of Asian type have replaced previously prevalent A prime or FM1 type influenza A(1-3). Clinically, infection with Asian strains has been relatively mild(1,3) although extremely high incidence of influenza at the peak of the pandemic did result in significant increase in influenza and pneumonia mortality. Availability of Asian strain isolates provided an opportunity to study biological properties previously employed in characterizing other influenza viruses and to compare these characteristics (1) with earlier A strains and (2) Asian strains recovered from mild and fatal cases of human infection. Asian strains were compared to PR8 and FM1 strains in relation to

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greater or less cytotoxicity(4), neurotoxicity (5), capacity to induce xanthine oxidase activity in infected mouse lung(6), and neuraminidase activity(7). As reported previously (1), hemagglutination titers (HA) of allantoic fluids from eggs infected with Asian strain viruses were consistently lower than titers of PR8 and FM1 strains. Certain of the early Asian isolates appeared to have a greater toxicity for embryonated eggs than did most A strains. This report presents evidence indicating that relationships between infectivity and hemagglutinin activity and cytotoxicity, neurotoxicity, and capacity to induce xanthine oxidase activity were not significantly different among Asian and other strains of influenza A tested. However, Asian strains did show a significantly greater neuraminidase activity to urine mucoprotein than PR8 and FM1.

Materials and methods. Viruses. Asian strain influenza isolates, designated as Q25, Q734, and Q548, were obtained from Dr. J. J. Quilligan, Jr., College of Medical Evangelists, and strain Grawley isolated in this laboratory. These strains were passed in eggs about 3 to 10 times. Prototype strain, Japan 305/57, was obtained from Dr. K. Jensen, Communicable Disease Center, Montgomery, Ala. Prototype strain FM1 was obtained from Amer. Type Culture Collection and strain PR8 was originally obtained from Lederle Laboratories by Dr. Carl De Boer. *Virus preparations.* Allantoic fluids from about 100 eleven-day-old chick embryos, inoculated with 10^{-3} or 10^{-4} dilutions of egg adapted viruses, were harvested, pooled, and concentrated. The pools were centrifuged in a 21 rotor in ultracentrifuge (Spinco model L) at 20,000 rpm for 60 minutes. The pellets were resuspended in about one ml of supernate/tube, then suspensions were ground in glass tissue grinder. This was followed by a short cycle of low speed centrifugation (International model V) to remove most of the tissue debris. Virus concentrates were stored in 2 ml vials in a –40°C deep freeze chest. The concentrates withstood about 2 months' storage without significant loss in activity. *Hemagglutination titrations.* Serial 2-fold dilutions of infected allantoic fluids were diluted in phosphate buffered saline beginning with 1:10 dilution. One-fourth ml of 1% suspension of washed human O cells in phosphate buffered saline was added to 0.5 ml of allantoic fluid dilutions. The tubes remained at room temperature for about 2 hours and the highest dilution showing complete or almost complete agglutination pattern was considered as end point. *Infectivity in eggs.* Ten-fold dilutions of infected fluids were inoculated into 4 to 6 eggs/dilution. Eggs were incubated for 48 hours, 37°C, chilled, and 0.5 ml of 0.5% human O cells suspension was added to 0.1 ml of test material. The tubes remained for 2 hours in cold room, 0-5°C, and observed for agglutination. Infectivity, EID_{50}, was calculated by the method of Reed and Muench (8). Toxicity of virus preparations to HeLa cells, obtained from Dr. J. T. Syverton, and to amnion cells (FL), obtained from Dr. J. Fogh, were carried out as described by Henle *et al.*(4). Toxicity of virus preparations to Swiss albino mice, strain Webster, males, 4 to 6 weeks old, were carried out as described by Henle and Henle(5). Xanthine oxidase activity of mouse lung homogenate was determined by the method of Sellers and Jann(6). Neuraminidase activity of infected allantoic fluid was measured as follows: One ml of various dilutions of infected allantoic fluids, diluted in pH 6.8, 0.025M phosphate buffer, was added to 4 ml of mg/ml phosphate buffer solution of urine mucoprotein prepared by method of Tamm and Horsfall(9). Enzyme reactions were carried out in airtight 50 ml Erlenmeyer flasks in a 37°C incubator and on a Kahn shaker for 18-24 hours. Reaction flasks were heated in a 56°C water bath for 30 minutes; the reaction mixtures were transferred to dialysis tubing and dialyzed against distilled water in cold, 0-5°C, overnight. Enzyme activity was determined by measuring the resorcinol reacting substrate(10) which was not dialyzable.

Results. HA titers of allantoic fluids of PR8 and FM1 were both 1:640 as compared to titers of 1:80, 1:160, and 1:320 for Asian strains (Table I, Fig. 1).

Attempts were made without success to prepare higher titers of the Asian strains in eggs by repeated passages and by inoculating various dilutions of the infected fluids.

TABLE I. Source, Serotype, Hemagglutination Titer, Infectivity, Cytotoxicity, Neurotoxicity, and Xanthine Oxidase Activity of Homogenates of 7 Influenza Strains.

Strain	Source	Serotype	HA titer* Pool	HA titer* Concentrate	EID_{50} of concentrates†	Cytotoxicity HeLa	Cytotoxicity FL	Neurotoxicity	Xanthine oxidase‡
				× 1000					
PR 8	Laboratory strain	A	640	40	7.8	3+	4+	+	2.2
FM 1	*Idem*	A′	"	40	6.6	4+	+	+	2.5
Japan 305/57	Egg line	Asian	160	10	7.8	+	2+	+	3.2
Grawley	Throat washing, mild case	"	"	5	7.0	2+	+	+	2.5
Q 25	*Idem*	"	80	5	6.8	2+	§	+	1.9
734	Lung, fatal case	"	160	5	7.0	4+	4+	+	1.7
548	*Idem*	"	80	10	5.6	4+	4+	+	2.5

* HA titers reciprocal of dilution. † Log/ml. ‡ Ratio of μl O_2 uptake of infected lung homogenate to control. § Q 25 not tested with FL.

+, positive response.

Several additional Asian strains isolated in this laboratory were initially tested. Allantoic fluid preparations of strains designated as R1, R2, R3, R4, and R5 had HA titers of 1:40, 1:80, 1:40, 1:20, and 1:40 respectively. HA titers of 3 additional strains obtained from Dr. Quilligan were all 1:20. The strains collected included strains from fatal and from mild cases. Because allantoic fluid preparations of the 12 Asian strains recovered in the Los Angeles area were neither neurotoxic nor cytotoxic, 4 strains listed in Table I were selected for concentration and more detailed studies. In addition, strains PR8, FM1, and Japan 305/57 were included in final study. Large pools of about 900 ml of infected allantoic fluid/strain were centrifuged to concentrate the virus particles.

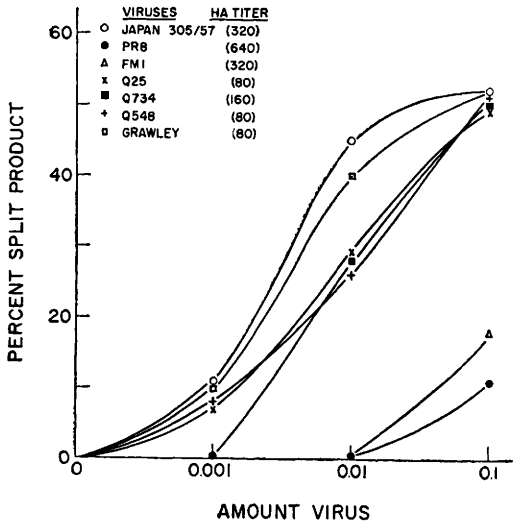

FIG. 1. Enzyme activity of 7 strains of influenza A viruses expressed as % resorcinol reacting compound split from urine mucoprotein. Each reaction flask contained the indicated volume in ml of infected allantoic fluids. The original HA titer of each fluid is listed.

In general, fluids with higher initial HA titers resulted in higher HA titer virus concentrates. However, the relatively low infectivity, EID_{50}, of virus concentrates of FM1 and Q548, log 6.6 and log 5.6 respectively, did not correlate well with HA titers (Table 1), possibly because of aggregation of particles and loss in infectivity during concentration. Crude growth curves did not reveal pronounced difference among strains as determined by EID_{50} and HA titers of the infected allantoic fluids. The EID_{50} of infected allantoic fluids of strains tested were fairly uniform, ranging from log 7.3 to log 8.0. HA titers of those strains were similar to titers given in Table I.

Four biological characteristics, cytotoxicity to cell cultures, neurotoxicity to mice, capacity to increase xanthine oxidase activity of infected mouse lung and neuraminidase activity of selected strains were determined, (Table 1, Fig. 1). Only virus preparations containing a relatively large number of particles were cytotoxic to HeLa and FL cell cultures. Cytotoxic effects were more pronounced in HeLa cell than in FL cell cultures and it was also observed that it was necessary to have virus preparations with HA titers of at least 1:1000 to 1:2000 to demonstrate cytotoxicity. The cellular changes observed in cell cultures were similar to those reported by Henle *et al.* (4). Various degrees of cellular destruction

and granulation were noted and scored according to the system described by the Henle *et al.*, (4).

Neurotoxicity to mice was demonstrated by inducing convulsions in mice by the twirling test(5). The lower limits in terms of HA units of virus preparations necessary to demonstrate toxicity were approximately 1:5000, which is in general agreement with that reported by Henle and Henle(5). Moreover, it was easier to induce convulsions with 4-week-old mice than with 6-week mice. Mice usually survived if convulsions failed to occur within 72 hours after intracerebral inoculation.

Sellers and Jann(6) reported that xanthine oxidase activities of infected mouse lung homogenates were approximately 2 times greater than normal lung homogenates. Enzyme activity was expressed as μl oxygen uptake. In this study enzyme activities of the 7 strains listed in Table I ranged from 1.7 to 3.2 times greater than normal homogenates. Endogenous activity has been accounted for in final calculations. The same amount of each virus concentrate was used to infect mice, without regard to HA titer of virus preparations and this did not seem to influence the extent of enzyme activity of the homogenates. Homogenates of lungs were prepared from mice inoculated intranasally 48 hours previously.

Neuraminidase activities of the 7 strains (Table I) are presented in Fig. 1. Enzyme activities are expressed as % resorcinol reacting split product, which was dialyzed against water, as calculated by difference, since in the actual analyses, the nondialyzable resorcinol reacting substrate was determined. On a relatively comparable HA titer basis, data presented in Fig. 1 show that the Asian isolates split off from about 10-40% more split product than PR8 and FM1. Normal allantoic fluid and/or phosphate buffer were added to control flasks and did not show any neuraminidase activity.

Discussion. The HA titers of the allantoic fluids of the 5 Asian type viruses were consistently lower than titers of PR8 and FM1. In addition, some Asian isolates were more toxic to chick embryo, suggesting that this difference in toxicity might be associated with some of the above characteristics. However, the 7 virus preparations did not show any pronounced qualitative differences in cytotoxicity, neurotoxicity, and xanthine oxidase activity on a comparable HA titer or infectivity basis. As shown by others(4,5), we also found that it was necessary to concentrate large pools of infected allantoic fluids to demonstrate cytotoxicity and neurotoxicity. These effects are believed to result from initial contact between a relatively large number of virus of virus particles and host cells. Moreover, according to Sellers(11), increased xanthine oxidase activity of infected lung is the result of a generalized host response. On the other hand, Asian influenza isolates consistently showed greater neuraminidase activity than PR8 and FM1. Whether the difference in activity is related to other biological or physical chemical properties is not known, but an increased capacity to attack mucoprotein virus inhibitors may have facilitated infection and pandemic spread of the Asian strains.

Summary. Asian strains of influenza A virus were neither more nor less toxigenic than PR8 and FM1 strains as determined by cytotoxicity, neurotoxicity and xanthine oxidase inducing activity. Asian strains did exhibit greater neuraminidase activity than PR8 and FM1. There were no differences in any of the above properties between strains from mild and fatal human infections.

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Dietary Glycine and Taurine on Bile Acid Conjugation in Man.*† Bile Acids and Steroids 75. (24741).

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Common bile acids occurring in human bile, *i.e.*, cholic, chenodeoxycholic and deoxycholic acids, are conjugated with glycine and taurine(1), glycine conjugates normally predominating. Thus, in 21 healthy medical students, a mean value of 3.2 was obtained for the ratio of glycine to taurine conjugates,§ and no free bile acids could be found. Bergström and Gloor(2), studying conjugation of cholic acid in homogenates of human livers, found that addition of 3 moles of taurine/mole of cholic acid caused pronounced increase in the proportion of taurine conjugates formed. Glycine did not have such a marked effect. These findings have been confirmed by Ekdahl(3). In our investigation, the *in vivo* effect of taurine and glycine has been studied.

Materials and methods. Two female and 9 male medical students were used. They were told to retain their ordinary diet, eat regularly, and to avoid excesses of alcohol intake during experiment. Taurine‖ (Eastman, puriss.), or glycine (Merck, for medical use) was given them with their meals 3 times a day for 5 days. Three other females with stones in roentgenologically normally functioning gallbladder were given taurine 3 times a day for 3 weeks. One subject (A.K.) had cancer in the deep bile ducts causing obstructive jaundice. Therefore, a T-tube had been inserted into the common duct so that one leg passed above the obstruction. After some weeks drainage was closed so that bile passed into the duodenum. The drainage had been closed for some weeks and the patient was at home with no jaundice when our experiment was done. Taurine was given 3 times a day for 5 days. Before and during taurine feeding, bile samples were taken through the drainage, which was opened for about one hour at noon every day. The samples were kept in refrigerator and frozen and stored at –16°C as soon as possible. In the other subjects, duodenal contents were collected through a polyvinyl tubing(4) which was introduced through the nose. Samples were taken at a level of 70-80 cm from the nose and the subjects were fasting on day of collection. Samples were immediately frozen at –16°C. Bile acids were analyzed according to Sjövall(5). Glycocholic, taurocholic, glycochenodeoxycholic and glycodeoxycholic acids were determined separately, whereas taurochenodeoxycholic and taurodeoxycholic acids were determined together.

Results. Fig. 1 shows the effect of orally administered taurine on conjugation of bile acids in the patient with a "circulating bile fistula." Prior to taurine feeding, glycine conjugated bile acids constituted between 65 and 75% of total bile acids. The day after administration of 3 g of taurine, equal amounts of glycine and taurine conjugates were found. Taurine was given 5 days and dose was increased by 3 g every day. On sixth day 96% of bile acids were taurine conjugated. Traces of free bile acids were found

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§ To be published.

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