patterns differ considerably even from one strain to the other of the same specie(2,3, 34).

Summary. The pentose pathway is the main route for carbohydrate oxidation in *Past. multocida* as demonstrated by C^{14} distribution after oxidation of labeled glucose, and the activity of the respective enzymes in cell free extracts from the organism. The Krebs cycle operates as a supplementary oxidative mechanism. Glucose carbon is also utilized for synthesis of amino acids.

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Susceptibility of a Strain or Rat Virus to Statolon and Other Virus Inhibitors.* (28944)

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Antiviral substances vary in relative breadth of activity from interferon, whose scope encompasses agents as small as the enterovirus group(1) and as large as psitta-

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cosis(2), to certain plant extracts which discriminate among apparently closely related ECHO viruses(3). Intermediate are other compounds such as 5-fluoro-2′deoxyuridine (or FUDR) and 2-(1-hydroxybenzyl) benzimidazole, which are active against certain groups of viruses, leading to their being proposed as aids to identification and classification(4,5). In this latter regard a recently isolated strain of rat virus has been studied, not only to relate it to other viruses in terms of comparative susceptibilities to inhibitors, but also to explore the antiviral spectrum of statolon. The inhibitors selected included statolon, an antiviral antibiotic(6); FUDR, reported to inhibit DNA viruses(4); and guanidine, reported to inhibit certain RNA viruses(7,8).†

Materials and methods. Rat virus, strain X-14, propagated in rat embryo cell cultures, was used. This virus was isolated from the mammary tissue of an X-irradiated Sprague-Dawley rat(9). Hemagglutination inhibition tests show it to be related to Kilham's rat virus and Toolan's virus, strain H3, members of a recently characterized group of viruses (10). Virus was assayed by hemagglutination of guinea pig erythrocytes or by production of cytopathology in rat embryo cell cultures (10). Unpublished data from this laboratory indicate that hemagglutinin is associated with the virus particle *per se*. Secondary rat embryo cell cultures were grown in stationary tubes in modified Eagle's basal medium (twice the usual amount of amino acids, vitamins, and glutamine) and 10% heat-inactivated calf serum. When cultures were to be used for infectivity titrations and inhibition experiments the medium was changed to contain 5% calf or fetal calf serum. Antiviral agents in amounts equal to or less than the maximum tolerated concentrations, as judged by cell morphology, were added to cell culture 24 hours before inoculation of an estimated 100 $TCID_{50}$'s of virus. The medium, including an appropriate concentration of antiviral agent, was replaced on the fourth day following infection. After cultures were observed for 11 days, the virus yield in the frozen-thawed cultures was measured.

† Statolon (Lot 209-493B-141-1E) and FUDR were generously supplied by Lilly Research Laboratories and by Hoffmann-LaRoche, Inc., respectively. Guanidine hydrochloride was purchased from Eastman.

TABLE I. Chemical Inhibition of Replication of Rat Virus.

Treatment	Concentration	Virus yield, HAU/ml
Exp No. 1		
None	—	2,560
Statolon	1:100 (v/v)	<10
"	1:200	<10
Guanidine hydrochloride	500 μg/ml	<10
" "	250 μg/ml	<10
Exp No. 2		
None	—	5,760
Statolon	1:400	80
"	1:800	20
Guanidine hydrochloride	500 μg/ml	<10
" "	250 μg/ml	2,080
FUDR*	10^{-6}M	<10
"	10^{-6}M	360
Thymidine	10^{-5}M	12,800
FUDR + thymidine	10^{-6}M + 10^{-5}M	11,520

* 5-Fluoro-2′-deoxyuridine.

Results. Table I presents data to show that the production of rat virus in infected cultures was inhibited by statolon, guanidine, or FUDR; and that inhibition by FUDR was prevented by thymidine. Samples from uninfected control cultures did not produce hemagglutination confirming their freedom from contaminating virus. In addition, since a decreased level or the absence of hemagglutinin could reflect either the absolute amount of hemagglutinin or the performance capability of the indicating system, the competency of samples from inhibitor-treated cultures to hemagglutinate was verified by adding virus to aliquots from cultures treated with the highest concentration of each inhibitor. Although in the first experiment clear-cut viral cytopathology was not observed, a change from calf to fetal calf serum in the second and subsequent experiments consistently resulted in manifest viral cytopathogenicity. Infectivity measurements on samples from the first experiment showed that the virus yield from untreated cultures was $10^{5.5}$ $TCID_{50}$'s per ml, whereas no virus was detected ($10^{0.5}$ $TCID_{50}$'s or less) in ali-

quots of cultures treated with statolon or guanidine hydrochloride. Inhibition of development of viral cytopathology paralleled inhibition of virus production in the second experiment. The relatively healthier condition of cells in the second test may account for the lesser effect of guanidine hydrochloride at the lower concentration, since unusually large cell populations in tubes of the first test may have resulted in suboptimal vigor.

Discussion. The study of rat virus and statolon in conjunction with other inhibitors is of interest not only for its implications in regard to potential chemoprophylaxis or chemotherapy but also for its contribution of data pertinent to the classification of strain X14 rat virus and the assessment of its significance. Inhibition by FUDR has been proposed(4) and accepted(11) as a test for the presence of deoxyribonucleic acid in a virus. Inhibition of replication of strain X14 by FUDR and protection by thymidine indicate that the viral nucleic acid is DNA. This is in agreement with the finding cited by Toolan (12) of extractable, infectious DNA in tissues containing a related virus. The antiviral spectrum of guanidine, initially reported limited to enteroviruses(7), later was reported to include measles(8). The present study suggests a DNA virus susceptible to guanidine. The significance of this finding is difficult to assess, however, because until one can discriminate between intrinsic resistance as a species characteristic and induced resistance due to previous contact, the alacrity with which viruses develop resistance to guanidine(13,14) leads to reservations about its utility as an aid to classification.

The viruses susceptible to statolon or the closely related if not identical M5-8450(6) include arbor viruses(15); picorna viruses such as polio-(16,17), other entero-(18,20) and rhinoviruses(21); as well as Rous sarcoma(22) among RNA-containing viruses. DNA viruses susceptible to statolon are the rat virus, according to the present study; polyoma (Johnson, I. S., personal communication); and probably SV_{40}, according to preliminary results by Hull (personal communication) and by ourselves. There is no direct evidence that viruses related to strain X14 produce tumors(10), but its isolation from a host with an increased probability of developing a tumor, as may be deduced from the prior exposure of the host to radiation, and the oncogenic nature of other DNA-viruses susceptible to statolon may constitute circumstantial evidence. Size determinations by electron microscopy(9) show that rat virus has a diameter of approximately 22 mμ. The size and apparent nucleic acid composition of rat virus, considered in relation to other viruses susceptible to statolon, suggest that the spectrum of statolon activity may be related to virus size or complexity, including both DNA and RNA viruses within that size range.

Summary. Rat virus, strain X14, a small (22 mμ) virus isolated from the mammary tissue of an X-irradiated rat, was found to be inhibited by statolon and by guanidine. Since statolon, a penicillium-derived antibiotic previously had been found to inhibit RNA viruses of the arbor- and entero-groups, it is noteworthy that rat virus was also inhibited by 5-fluoro-2-deoxyuridine and that the inhibition was prevented by thymidine, a conventional identification of a DNA virus.

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Bleeding Volume Indices of Hydroxyethyl Starch, Dextran, Blood, and Glucose.* (28945)

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Intravenous administration of selected variations of hydroxyethyl starch in dogs has indicated that their blood stream persistence and tolerance reactions are of the same order as dextran(1). A high grade of tissue compatibility might be expected particularly in the instance of amylopectin which is structurally more closely related to glycogen than any other glucose polymer(2). In experiments with starch as a plasma substitute in World War II, there was no evident means of preventing rapid enzymic degradation of this colloid polymer. Currently, a controlled degree of enzyme resistance is obtained on an industrial scale by introduction of hydroxyethyl groups through interaction with ethylene oxide and alkali. The present series of studies has led to the selection of a "waxy" maize starch consisting largely of the highly branched amylopectin with minimal content of the linear, amylose fraction(3), acid-modified to a stage of low viscosity (specified industrially in this case as 70 or 90 fluidity) and, after gelatinization, derivatized to 0.7 degree of substitution (hydroxyethyl groups per anhydroglucose unit). These and a number of related products were obtained through the cooperation of Drs. T. J. Schoch and E. F. Paschall of the Corn Products Co., Argo, Ill. Biologic variables which have been tested with these products in over 400 experiments have included hemodynamic, respiratory and renal function changes, bleeding times, acid base balance, sedimentation rates, plasma protein values, histamine release and tissue storage(1,4,5). One of the most critical characteristics in terms of possible usefulness is the bleeding volume index. The present study was designed to compare the "bleeding volume indices" of hydroxyethyl starch and dextran as well as that of blood and glucose. This procedure first described by Gordon *et al*(6) represents a simple but significant means of analyzing the relative efficacy of volemic agents in hemorrhagic hypotension.

Methods. Thirty-nine mongrel dogs of both sexes weighing 9.0 to 14.4 kg were anesthetized with 15 mg/kg of sodium pentobarbital and 225 mg/kg of sodium barbital intravenously, a combination producing stable anesthesia for at least 4 hours. Polyethylene catheters were inserted into both femoral arteries and veins and advanced to the thoracic level. Aortic pressures were measured with Statham transducers through one arterial catheter and the contralateral arterial

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