ployed in these experiments was well within therapeutic limits. This is of some practical significance, as these findings may help to explain the constipation which sometimes develops in patients taking Dilantin.

These results may also be of importance in relation to the problem of so-called "abdominal" epilepsy. Commonly accepted criteria for the diagnosis of this form of seizure include: (1) episodic attacks of abdominal pain, (2) "cerebral dysrhythmia" as revealed by electroencephalography, and (3) improvement with marked reduction or cessation of these attacks upon the institution of anti-epileptic medication, usually with Dilantin. The implication has been that improvement upon the institution of this medication is due to an effect upon the central nervous system. The possibility of such improvement being due to a direct effect of the medication upon the gut has not been given adequate consideration. If, as has been demonstrated in these experiments (*in vitro*), the Dilantin has a direct effect upon the gut (*in vivo*), then the third criterion is no longer necessarily valid in indicating the "central" origin of these attacks. The problem of "abdominal" epilepsy should be considered without regard for the effects of anti-convulsant medication, unless the direct effects of this medication upon the gut can be dissociated from its effects upon the central nervous system.

These experiments may have clinical implications in other diseases affecting the gastro-intestinal tract, particularly those where increased motility or spasticity, or both, are prominent.

Summary. (1) Dilantin has a direct effect upon isolated rabbit gut. It reduces tone, reduces the amplitude and rate of contractions, and in higher concentrations can completely abolish the contractions. (2) The effect of Dilantin upon the isolated gut is rapidly reversible by simply replacing the gut in fresh Tyrode solution. (3) This effect of Dilantin is not due to its alkalinity. (4) Dilantin has a much more marked effect upon the gut than has sodium phenobarbital. (5) The clinical implications of these effects of Dilantin upon isolated gut are discussed briefly.

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Propagation of Equine Abortion Virus in the Chick Embryo.* (21973)

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Doll(1) was the first to offer unequivocal proof that the virus of equine abortion (EAV) can be cultivated in the chorio-allantoic membrane of the chick embryo with intranuclear inclusions characteristic of the disease. The hamster-adapted variant used in his experiments would not induce infection in chick embryos by allantoic, amnionic or yolk-sac inoculation. Doll has pointed out that all attempts at propagation with infected equine fetal tissue have met with failure in the chick embryo. Previous experience(2) has shown that a field strain of EAV can survive 8 serial passages in chick embryo lung and liver maintained in flasks. No inclusions could be identified in chick tissue but were demonstrated in fetal horse tissue inoculated with chick embryo passage material. In the present communication, the serial propagation of EAV in chick embryos is reported.

Material and methods. The virus used to initiate this study (strain F) had been adapted to the hamster liver(3). The liver inocula, hamster or chick embryo, were frozen and ground, diluted 1:10 in physiological saline and centrifuged lightly. Fertile eggs

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of 12-day incubation were injected with 10% liver suspension in the yolk and amniotic sacs with 0.5 ml and 0.1 ml respectively. Initially, the eggs were inoculated with hamster liver, and subsequently with chick embryo liver for 14 consecutive passages. Each serial passage was terminated 4-5 days following inoculation. In order to determine the pathogenicity of chick embryo material, alternate passages were inoculated into 3-week-old hamsters. Representative pieces of liver from the hamster and chick embryo passages were fixed in Zenker-acetic acid for histological studies.

Complement-fixation methods. The original complement-fixation procedure(4) for equine abortion employing the 50% hemolysin end-point method, was abandoned because of the excessive amount of reagents required. Doll later employed the conventional method(5). The latter procedure was used in the present study to save materials. The liver antigens were ground in physiological saline and stored at −50°C. Prior to use they were centrifuged at 2500 rpm for 10′. The origin and preparation of the positive and negative sera, and control antigens, have been previously documented(3,6). Essentially, the procedure utilized 2-fold 0.25 ml serial dilutions of antigen, 0.25 ml of 1:10 dilutions of antiserum of equine origin and 2 full units of complement in 0.5 ml. Fixation was carried out overnight at 4°C and 0.5 ml of sensitized cells were added for 1 hour at 37°C. Adequate controls were used for each reagent, including normal horse serum.

Results. Serial propagation in chick embryos. No significant mortality was observed in any of the trials. The search for intranuclear inclusions characteristic of the infection in susceptible species was unrewarding in early experiments. In the 6th and subsequent passages intranuclear inclusions were identified in liver parenchymal cells. They were indistinguishable from those seen in the horse(6). Inclusions were occasionally numerous, usually focal in distribution and not abundant.

Identification of embryo propagated virus. Hamster adapted equine abortion virus is quite pathogenic for young hamsters with LD_{50} titres varying between 10^{-6} and 10^{-8}.

TABLE I. Complement Fixation Reactions.

Antigens	Antigen titration end-points
Equine fetal lung (abortion virus)	128
Normal fetal lung	4
14th passage chick embryo liver	Anticomplementary
Normal chick embryo liver	"
Hamster liver inoculated with 14th passage chick embryo	64
Normal hamster liver	8

Intranuclear inclusions are constant and abundant in the parenchymal cells of the liver. Chick embryo passage material remained pathogenic for 3-week-old hamsters, but the results were variable. Ten per cent chick embryo liver rarely killed all inoculated animals. The liver from all dead animals contained abundant typical inclusions. Doll(7) reports that chick embryo EAV loses pathogenicity for weaned hamsters. The dilution of the original hamster material during serial passage in chick embryo exceeded 10^{-15}. The dilution is only approximate since the routes of inoculation preclude accurate calculation. The known limiting dilution of infectivity was greatly exceeded, showing that the agent has been propagated in the chick embryo. The chick embryo adapted virus was further identified by the complement-fixation reaction. The data shown in the table present proof that the specificity of the egg adapted virus was maintained. The antigen titration end-point is defined as the highest dilution of antigen which resulted in complete fixation of complement with a 1:10 dilution of antiserum of equine origin.

Summary. The adaptation of hamster modified equine abortion virus to the chick embryo is reported. Characteristic intranuclear inclusions were identified in the liver parenchymal cells of chick embryos and hamsters receiving serial passage chick embryo liver. Identification of the virus is substantiated by the use of the complement-fixation reaction.

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Determination of Specific Activity of Sodium in Bone.* (21974)

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Studies of the role of skeletal sodium have been hampered by technical difficulties encountered in the analysis of this tissue for sodium content. These difficulties arise largely from the fact that bone contains tremendous amounts of calcium, the molar Ca/Na ratio being of the order of 25 1. This quantity of calcium causes serious interference with both the flame photometric and gravimetric methods for determination of sodium; and phosphate, in the quantities present in bone, may also interfere. Radioactivity measurements require large corrections for self-absorption if unaltered solutions of bone ash are used, and if attempts are made to precipitate calcium and phosphorus, as much as 20% of the radiosodium present may be removed by co-precipitation(1).

In an attempt to circumvent the difficulties inherent in analysis of bone for sodium, a new method was developed in which interfering substances are removed by cation exchange chromatography(2). Under the conditions employed, sodium appears in the eluate entirely free from phosphate, calcium, magnesium and potassium, whereupon the sodium-containing fraction can be easily analyzed by flame photometry.

The present report extends this investigation to include the behavior of radioactive sodium on the column, and further defines the accuracy and limitations of the method.

Method. Column characteristics — A column 1.3 x 60 cm is employed, containing 27 g (oven-dried basis) of the sulfonated polystyrene resin Dowex 50-x12 (50-100 mesh). The resin rests on a plug of Pyrex glass wool and fills the column to a height of 42 cm. Details of the preparation and operation of the column and the collection of eluate fractions are given in a previous publication(2). *Analysis of Effluent Fractions.* Analyses for Na^{23} and for K and Mg were carried out by standard methods (for references see 2). Calcium was determined by the hexanitratocerate technic (3). For purposes of this study, a special hood was fitted to the Weichelsbaum-Varney flame photometer in order to exhaust radioactive fumes to the outside. Measurements of Na^{22}† were made by pipetting 2 ml samples of eluate into 1.5 x 4.5 cm vials, which were then counted directly in a well-type scintillation counter. In instances where the activity was low, the sample was first evaporated on a steam bath to the appropriate degree of concentration. A known aliquot of Na^{22}, appropriately diluted in NaCl solution, served as a standard. Decay corrections were obviated by counting the eluate fractions and the standard solution on the same day. The usual corrections for coincidence effects were applied. *Preparation of Bone Samples.* Bone is first dried and then ashed overnight at 525°C in a muffle furnace. The ash is dissolved in 3 N HCl, and the resulting solution boiled for 10 minutes. This solution is then placed on the column, any remaining dark particles having been removed by filtration through Pyrex glass wool, allowed to flow into the resin bed, and elution with 0.8 N HCl be-

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