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Effects of Sodium Diphenylhydantoinate upon Isolated Small Intestine of the Rabbit. (21972)

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The effects of a number of drugs upon the rhythm of isolated rabbit intestine were studied. Among the drugs tested was sodium diphenyl-hydantoinate (Dilantin). This proved to have remarkable effects which, to our knowledge, have not been described previously.

Material and methods. The small intestine was rapidly removed from a freshly stunned rabbit. This was immediately placed into oxygenated Tyrode's solution. Lengths of duodenum were most generally employed, although jejunum and ileum were also used in some experiments. The unused portions were stored in cold Tyrode solution for later use. The length of the gut used was between 2 to 3 cm. One end of the gut was fixed by a silver hook which in turn was fastened to the walls of the chamber. The other end of the gut was led by a thread to a very sensitive myograph transducer. This in turn was connected to the amplifier of a Hoff-Geddes-Spencer Physiograph and the resultant recording was traced out by an ink-writing pen upon a moving strip of paper. The amplification factor for movement was about 200 at maximum gain. With the initial tension of the myograph spring overcome, a 200 mg weight produced a deflection of 7.5 mm at maximum gain.

The chamber enclosing the isolated gut had 3 openings at its base. The first of these allowed for the continuous entry of oxygen into the solution. This kept the solution well-oxygenated and also served to mix the solution and any added drugs. An opening at the lowest part of the chamber allowed for drainage at will and a third opening provided for the entry of fresh Tyrode solution of pH about 7.6. A further portal in the upper part of the chamber enabled one to add drugs to the solution. The bath was kept at any constant temperature between 35° and 38°C by means of a heat lamp which was shone upon a blackened portion of the chamber. The intensity of this heating was regulated by means of a variac.

Results. The addition of Dilantin in amounts sufficient to produce a resultant concentration of 2 to 8 mg% (0.07 to 0.29 mmol/l) in the solution resulted in a rapid decrease in the amplitude of the contractions and a fall in tone (Fig. 1, A and B). This effect was noted within 15 to 30 seconds and the contractions generally disappeared within one minute. The addition of smaller amounts of Dilantin resulted in a more gradual decrease in the tone and in the amplitude of the contractions. A concentration of 0.5 mg % (0.018 mmol/l) was sufficient to reduce considerably the amplitude of contraction of the gut (Fig. 1, C). With the smaller concentrations of Dilantin, the gut contractions would become reduced in

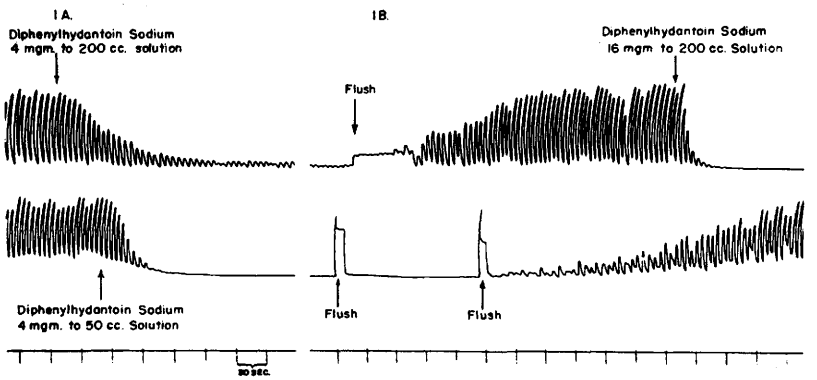

FIG. 1. A. Effects of Dilantin upon 2 separate samples of rabbit duodenum, both examined simultaneously. Note rapid decrease in amplitude of contractions and fall in tone upon addition of the drug; also note more rapid effects of higher concentrations. B. Note rapid return of contractions of normal amplitude upon flushing away the Dilantin. Gut exposed to higher concentration requires a longer time and more flushing before returning to normal level of contractions and of tone.

amplitude, but after several minutes the contractions would become fairly constant in strength.

Dilantin affects the rate as well as the amplitude of contractions. This effect is apparent in concentrations of the drug which markedly depress the amplitude of contractions without, of course, abolishing them (Fig. 2). By means of increasing the amplifying factor of the apparatus, it was possible to detect minute contractions of the gut. With concentrations of Dilantin of 6 to 8 mg%, the rate of contractions was reduced to one-third to two-thirds of the control rate.

The effects of Dilantin are rapidly reversible. Even after the gut had been exposed to concentrations of the drug sufficient to prevent any recordable contractions over a period of over 50 minutes, removing the drug solution and replacing it with fresh Tyrode solution led to the appearance of small contractions within 75 seconds and a return to normal amplitude of contractions within 3 minutes. This reversibility of effect was present after repeated exposures to the Dilantin over a period of 8 hours.

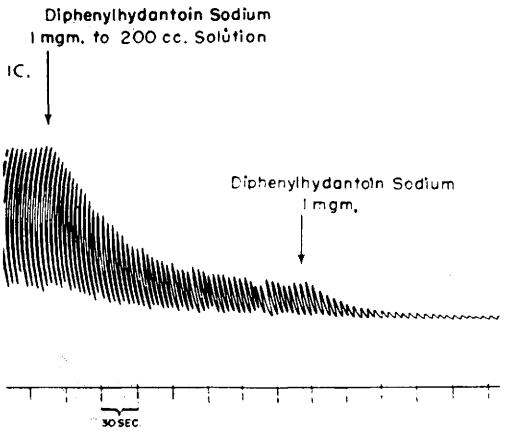

FIG. 1. C. Slower and lesser effect of smaller concentrations of Dilantin on amplitude and rate of contractions.

To rule out the possibility that any of the effects noted were due to the alkalinity of the Dilantin, sodium hydroxide solution was added to the Tyrode solution bathing the gut in amounts sufficient to equal and, in some instances, exceed the pH of a solution of Dilantin concentrated enough to stop the contractions of the gut. Such an addition of base led to an increase in tone (Fig. 3A)(1). This increase in tone could be reversed and the tone returned to normal levels by the addition of Dilantin to the already alkaline solution (Fig. 3B). This would appear effectively to rule out simple alkalinity as the basis for the mechanism of action of the Dilantin.

The effects of the Dilantin were compared with the direct effects of sodium phenobarbital upon the isolated gut. In concentrations of 10 mg% (0.39 mmol l), the sodium phenobarbital had a slight effect in reducing the rate of contraction from 16 per minute to 14

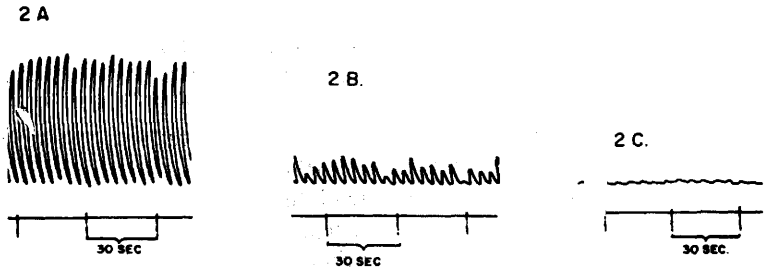

FIG. 2. A. Control record of contractions of gut. Note great amplitude of contraction and rate of 16 per min. B. In intermediate concentrations of Dilantin, amplitude of contraction is reduced. Rate is 15 per min. Amplification has been increased. C. In a concentration of 6 mg % Dilantin contractions of the gut are barely visible, even with the maximum gain of the amplifying system. The rate is 11 per min.

per minute, with some reduction in the amplitude of contraction as well. At a level of 21 mg% (0.83 mmol/l) there was a marked reduction in the amplitude of contractions and the rate was 14 per minute. The effects of the sodium phenobarbital upon the gut were much less rapidly reversible than those of Dilantin.

Discussion. A concentration of 0.5 mg% of Dilantin was effective in reducing the amplitude of contraction of the isolated small intestine of the rabbit. Concentrations of 8 mg% abolished recordable contractions.

It has been reported(2) that the blood levels of hydantoinate in human patients receiving Dilantin for the treatment of epilepsy range between 3 to 6 mg%. Thus, it is obvious that the range of concentrations em-

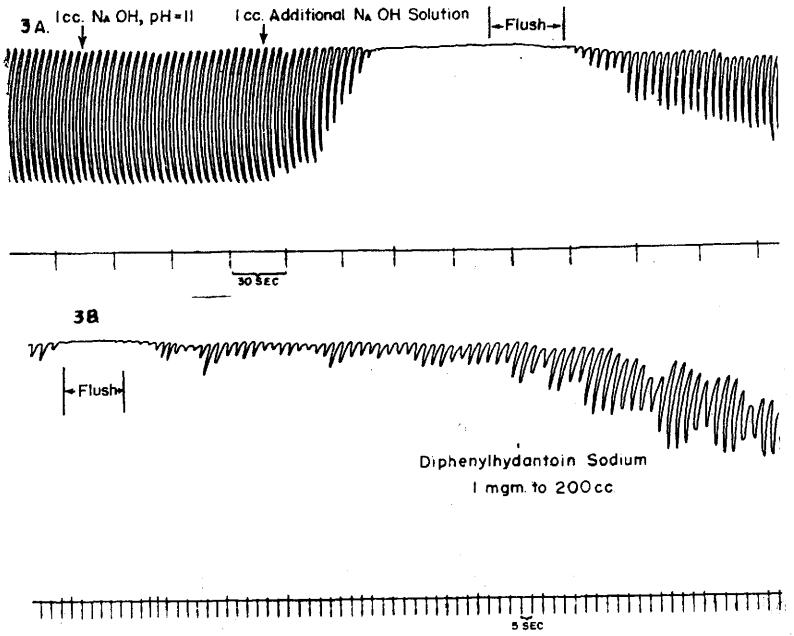

FIG. 3. A. Rise in tone upon addition of NaOH solution. Although the record suggests tha contractions stopped with the increase in tone, this was not so. Gut continued contracting, bu contractions were not recorded since normal tension range of this myograph was exceeded. This effect of NaOH is reversible by flushing. B. Flushing after addition of base leads to a slight reduction in tone. Addition of Dilantin leads to rapid fall in tone. Apparent increase in amplitude of contractions is artefactual and is due to tension exceeding recording range of the myograph in the earlier section of the record.

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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