Results. Table 1 illustrates the difference between groups of male and female rats which received subplantar injections of 1 mg of carrageenin. The time-course temperature profiles of untreated male and female rats repeatedly demonstrated an early-phase drop in body temperature in male groups which was not seen in female groups. The typical carrageenin-induced pyretic trends in male and female groups may be reversed by pretreatment with estradiol in males and testosterone or ovariectomy in females. It was also found that pretreatment of male rats with testosterone and female rats with estradiol resulted in a slightly depressed pyretic pattern as compared to the respective non-pretreated groups. Pyretic studies in the estradiol-pretreated females were carried out at the state of full estrus as determined by microscopic examination of vaginal washings according to the Doisy method[6]. Non-pretreated females were found to be in random stages of the estrus cycle on the day of carrageenin testing while vaginal smears from females pretreated with testosterone resembled a typical castrate or modified diestrus stage. Table 2 summarizes the over-all peak in temperature increase seen in the various experimental designs used in this study.

Table 1. Average increase per group in rectal temperature of rats promoted by subplantar injection of 1 mg carrageenin

Type of carrageenin	Peak rise in temperature (°C) from pre-injection value	
	Male	Female
Lambda	0.63	1.37
Viscarin	0.42	1.59
Gelcarin	0.23	0.57
Kappa	0.20	0.26
Iota	0.24	(–) 0.28

Peak temperature rise invariably occurred at 5–7 h post injection.

Table 2. Changes seen in the average peak temperature increase in groups of male and female rats under different experimental pretreatments

Experimental Design/pretreatment	Peak rise in temperature (°C) from pre-injection value
Females/(no pretreatment)	1.59
Males/(no pretreatment)	0.42
Females/testosterone	1.00
Males/estradiol	1.43
Ovariectomized	0.55
Males/testosterone	0.22
Females/estradiol	1.17

These values summarize the hormonally-modulated 'reversal' trend.

Discussion. This study demonstrates that subplantar injections of 1 mg carrageenin causes an increase in core body temperature which closely correlates with the inflammatory efficacy of the type of carrageenin used. The generalized pyretic response which reaches a maximum at 5–7 h after subplantar injection is more pronounced in intact females than it is in males. The time-course pattern of this hyperthermic response is in some manner dependent upon sex hormones. Select pretreatment schedules resulting in male/female hormonal modulation can alter the typical pyretic profiles such that the male and female pyretic patterns tend to 'reverse'. The relationship between the local inflammatory insult and the pyretic response is not entirely clear as it has been found by Sobanski et al.[4] that pretreatment with hydrocortisone reduces the local edematous response but does not prevent the onset or development of the hyperthermia. The biologic mechanisms whereby the female hyperthermic response surpasses that of the male remain to be elucidated. According to our data it is an interesting observation, however, that the endogenously regulated stage of estrus in the intact female does not seem to play a major role in that carrageen pyresis is a most dramatic and reliable event in random-cycle animals.

6 Therapeutic Notes, Parke Davis & Co., p. 47 (1937).

Modification of clonazepam anticonvulsive activity by its association with other anti-epileptic drugs

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Summary. The modification of the anti-epileptic activity of clonazepam by other anticonvulsivants is studied. The results vary according to the drug and technique used. The usefulness of the 2 techniques employed is discussed.

Although the first derivatives of dibenzoazepines and dibenzodiazepines were not developed as anti-epileptic drugs but only as tranquilizers and hypnotics[1], they showed that chlorodiazepoxide, diazepam and nitrazepam reduce the thalamic epileptogenic excitability of the cat without reducing it in the cortex, while phenobarbital reduces both.Recently a new benzodiazepine, clonazepam, has been introduced. The difference with nitrozepam is an atom of chloride in position 2 of the benzenic ring. Swinyard and Castellion[2] have studied the experimental pharmacology and more recently Vuillon-Cacciuttolo and Issautier[3] have studied the electroencephalographic effects and found that it has barbiturate-like properties, with the exception of the hypnotic activity. Gastaut et al.[4], as well as Poire et al.[5], have undertaken trials in humans with clonazepam and have seen that the product is useful in all types of epilepsy in adults.

The various types of convulsive syndromes, especially major epilepsy, are generally treated with combinations of different anti-epileptic drugs[6]. This is the reason why we undertook the present experiment. Our aim has been to study the modification of clonazepam anticonvulsivant activity by other anti-epileptic drugs such as dipropylacetic acid, phenobarbital, mephobarbital, diphenyl-

hydantoin, phenylethylacetylurea, sulfthiame, diazepam and tiadipone. From all the technique described for evaluation of the anticonvulsivant activity of the drugs[7], we selected the supramaximal electroshock for reproducing a syndrome like the epilepsy major in man, and the technique of cardiazol to reproduce all kind of epileptic syndromes of man[2], particularly epilepsy minor[8].

Material and methods. Male and female white mice of ICR Swiss strain were used. The drugs were administered by i.p. route. 2 technique were followed: induction of convulsions by cardiazol (80 mg/kg i.p.) and induction by maximal electroshock (100 Hz, 3 msec, 80 V, 0.3 sec of duration applied through ocular electrodes[9]. The drugs studied were dipropylacetic acid, phenobarbital, mephobarbital, diphenylhydantoin, phenylethylacetylurea, sulfthiame, diazepam and tiadipone[10]. The ED_{50} of each drug was determined for each of the technique used. Later the ED_{50} for clonazepam was determined and the modifications of the ED_{50} of the antiepileptic drugs were studied in presence of the ED_{50} of clonazepam with the 2 technique used. The drugs were administered 30 min before the convulsivant agent. The method of Litchfield and Wilcoxon[11] was used for the statistical calculation of ED_{50} and the test X^2 for comparison of activities[12]; the coefficient of activity was the quotient between the association activity and the clonazepam activity.

Results. The results are shown in the table and figure 1 and 2. Using cardiazol as convulsant agent, dypropylacetic acid, mephobarbital, diphepnlhydantoin and diazepam do not modify the activity of clonazepam while phenobarbital and phenylethylacetylurea do inhibit its activity and tiadipone and sulfthiame potentiate it. When the convulsant agent was an electroshock, dipropylacetic acid does not modify the activity, while phenobarbital, mephobarbital, phenylethylacetylurea, diphenylhydantoin, sulfthiame, diazepam and thiadipone potentiated clonazepam's anticonvulsive activity.

Discussion. The 9 anticonvulsive drugs used in this experiment can be divided in 4 groups: active in epilepsy major (phenobarbital, mephobarbital and diphenylhydantoin), in epilepsy minor (sulfthiame), in psychomotor accesses (phenylethylacetylurea) and in all types of epilepsy (dipropylacetic acid, diazepam, tiadipone and clonazepam)[13]. All these drugs, except dipropylacetic acid, potentiate clonazepam's anticonvulsant activity when a supramaximal electroshock is applied to the animals. In cardiazol-induced convulsions, the drugs which are active in all types of epilepsy (except dipropylacetic acid) and sulfthiame (specific for epilepsy minor) potentiate clonazepam's anticonvulsivant activity; on the other hand, the drugs which are active in epilepsy major and in psychomotor fits reduce clonazepam's anticonvulsivant activity. This suggests that cardiazol-induced convulsions could be equivalent to epilepsy minor of adults, which is in accordance with the results of Toman[8]. The supramaximal stimulation reproduces epilepsy major of man[7] but not other types of epilepsy which can become apparent with less frequent and less intense stimuli. This proves that the technique has a lower specificity, and it would explain why in our experiments

Anti-epileptic activity

Drug	ED_{50} Cardiazol	Electroshock
Clonazepam	27.7 mcg/kg	250 mcg/kg
Dipropylacetic acid	150 mg/kg	50 mg/kg
Phenobarbital	3 mg/kg	25 mg/kg
Mephobarbital	3 mg/kg	25 mg/kg
Diphenylhydantoin	20 mg/kg	5 mg/kg
Phenylethylacetylurea	14 mg/kg	26.7 mg/kg
Sulfthiame	273.9 mg/kg	97.5 mg/kg
Diazepam	5 mg/kg	9 mg/kg
Tiadipone	35 mg/kg	40 mg/kg

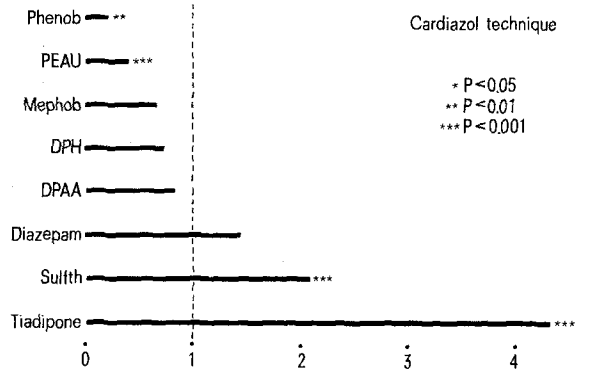

Fig. 1. Coefficient of activity of the association of clonazepam and other anti-epileptic drugs.

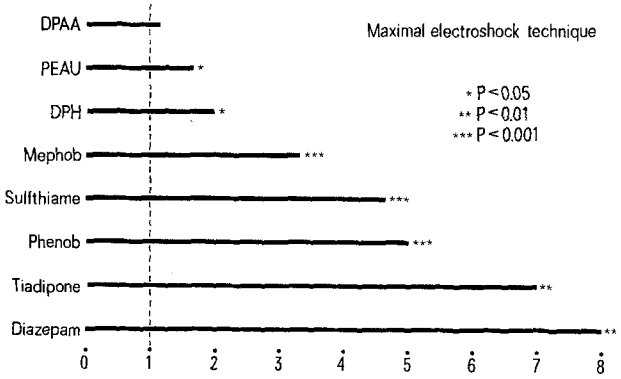

Fig. 2. Coefficient of activity of the association of clonazepam and other anti-epileptic drugs.

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clonazepam's anticonvulsant activity was potentiated by all the drugs tried. In any case, it has not been possible at present to find 'model animals' in which all kind of human epilepsy can be reproduced[14]. We believe that the different behaviour of all the drugs tried with clonazepam could be explained by the different mechanisms of action each of them has[13]. Barbiturates, phenurone and diphenylhydantoin have a common mode of action, although the first ones have a more sedative and depressive activity of the CNS than diphenylhydantoin. We have to point out that the doses of barbiturates and phenurone required to protect from the effect of maximal electroshock are higher than the doses required to protect from the effects of cardiazol; on the other hand, the ED_{50} of diphenylhydantoin required to protect against cardiazol convulsions is higher than the dose required to protect from the electroshock convulsions. This induces us to think that diphenylhydantoin posses certain peculiarities with respect to barbiturates which would explain why although they have similar antiepileptic activity, they behave dif ferently with respect to the anticonvulsant activity of clonazepam in convulsion–induced by cardiazol.

Therefore it can be concluded that: a) The cardiazol technique we have used induces particularly epilepsy minor crisis. This agrees with the results obtained by Toman[8]. b) The maximal electroshock technique is not specific for studying the effect of several associations of antiepileptic drugs. c) We believe that, although barbiturates and diphenylhydantoin have been grouped together as having similar mechanism of action and similar therapeutic indications, they have anticonvulsive peculiarities which can clearly be observed with the experimental technique used.

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The effect of verapamil and dibutyryl cAMP on the spontaneous activity of the sinus node

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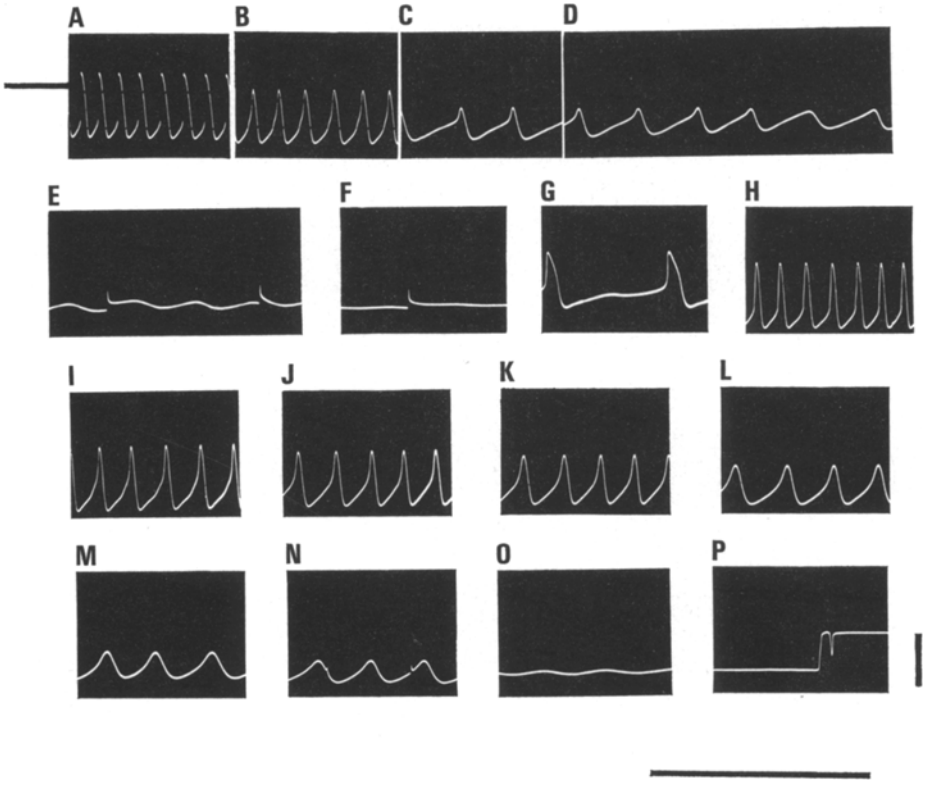
Summary. Verapamil stops the electrical activity of the sinus node cells. In the presence of verapamil, dibutyryl cAMP brings about a recovery of the spontaneous activity, whereas noradrenaline is ineffective.

Verapamil was initially thought to be a specific Ca-antagonist for smooth and cardiac muscle[1–4]. It has now been recognized that only (–)-verapamil exerts an inhibitory action on the slow Ca-channel, whereas its (+)-isomer interfers with the fast Na-channel[5, 6]. Thus racemic and (–)-verapamil can suppress the slow component of the action potential in myocardial cells[6–9]. The aim of present study was to investigate how verapamil acts on the electrical activity of the sinus node cell in the pacemaker region, and to find out if noradrenaline and dibutyryl cAMP (db cAMP) could counteract the verapamil effects. In these experiments, racemic verapamil was employed in a concentration that left the fast channel practically unaffected[6].

Methods. The experiments were performed on 24 isolated sinus nodes of the rabbit heart. Each preparation was



Effect of verapamil and db cAMP on the sinus node cell. *A* control, *B–F* action of verapamil, *B* 3 min, *C* 12 min, *D* 12 min 5 sec, *E* 15 min, *F* 18 min of perfusion; *G–H* db cAMP added to perfusion fluid containing verpamil, *G* 8 min, *H* 10 min of perfusion; *I–P* rinsing with Tyrode fluid containing verapamil, *I* 10 min, *J* 20 min, *K* 25 min, *L* 40 min, *M* 48 min, *N* 60 min, *O* 73 min, *P* 75 min of perfusion. In *E*, *F* and *N* the cell was stimulated. Calibration: voltage 50 mV, time 5 sec.