The strips were mounted in organ baths[4] containing Tyrode's solution (37 °C, pH 7.2) aerated with 95% O_2/5% CO_2. Contractile activity was monitored by Grass force-displacement transducers (FT O3C) and recorded by a Beckmann Dynograph. Strips were allowed to equilibrate at a resting tension of 0.5 g which was readjusted frequently until holding constant. At this tension, responses should be submaximal[4], and thus we hoped to detect response enhancement most easily. Addition of hormone solutions was done in small quantities, not causing any effects based on volume alone. Between drug additions, the bath fluid (19.75 ml) was changed 3 times and, after a period of 15 min rest, changed 2 more times. Further drugs were added after another 10 min of rest.

PGE_1 was kindly supplied by Dr. J. PIKE (Upjohn Co.) and kept as a stock solution of 2.85×10^{-6} *M*. Synthetic oxytocin (475 IU/mg) was kindly supplied by Dr. R. WALTER, Mt. Sinai School of Medicine, New Vork.

Results and discussion. Mammary strips from both pregnant and lactating rabbits responded to oxytocin. The former were about 100 times less responsive than the latter. In strips from pregnant rabbits, PGE_1 administration prior to oxytocin enhanced the contractile effect of the latter hormone (Figure 1). This increased response occurred when PGE_1 was added either 1 or 15 min prior to oxytocin. Higher doses of PGE_1 caused no further enhancement. The threshold dose of oxytocin was not altered by the presence of PGE_1.

In contrast, when strips from lactating rabbits were tested, PGE_1 administration caused a reduced oxytocin sensitivity (Figure 2). This effect was evident only after long-term exposure (15 min) to PGE_1. We also observed some degree of tachyphylaxis to oxytocin in strips from lactating animals. This allowed only 2 determinations per strip.

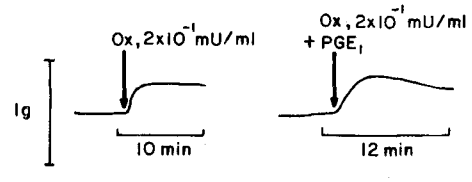

Fig. 1. Potentiation of the oxytocin response in a mammary strip from pregnant rabbit by PGE_1 (15×10^{-8} *M*).

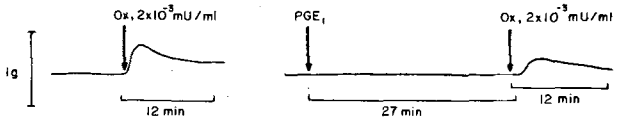

Fig. 2. Inhibition of the oxytocin response in a mammary strip from a lactating rabbit by PGE_1 (1.5×10^{-8} *M*).

Threshold doses for oxytocin were about 1.5 and 0.02 μU/ml in strips from pregnant and lactating animals, respectively; these values changed to 0.6 and 0.4 μU/ml after prior exposure of strips to about 1.5×10^{-8} *M* PGE_1.

The present study provides in vitro confirmation of the in vivo observations of an inhibitory effect of PGE_1 on oxytocin responsiveness of the lactating rabbit mammary gland[1]. Since this effect can be demonstrated on the level of isolated strips, it appears that PGE_1 acts directly on the myoepithelium and does not involve some preceding systemic event.

Since PGE_1 action is functionally opposite in strips from pregnant rabbits compared to strips from lactating animals, this suggests the existence of two different mechanisms of action of prostaglandins, one or both of which may be differentially expressed during periods of glandular development. Conceivably such developmental changes may occur either on the level of PGE_1 action (receptors) or PGE_1 metabolism. Both inhibition and enhancement or potentiation by prostaglandins of smooth muscle contracting agents has been described in the literature[6]. However, to our knowledge, opposing actions in the same organ as a function of its developmental or endocrine state have not been observed to date.

The fact that prostaglandins stimulate adenylate cyclase from mammary gland may be of relevance to the present observations. However, this hormone-sensitive enzyme was thought to be present in secretory cells[3], and it has not yet been established whether or not myoepithelial cells also contain a prostaglandin sensitive cyclase system. Considering the diverse roles of prostaglandins in reproductive physiology as well as the fact that this class of compounds is now in use for therapeutic purposes, it seems of interest to clarify further the possible physiological and pharmacological effects of these agents on the mammary gland.

Summary. PGE_1 administration to isolated strips from pregnant and lactating rabbit mammary gland resulted in different effects on oxytocin-induced contractions. In strips from pregnant animals, oxytocin action was enhanced; in those from lactating animals, it was reduced and threshold doses for oxytocin were markedly higher.

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10 June 1975.

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Antagonistic Effects of Pemoline to Colchicine and Caffeine

After former studies on the medicinal effects of pemoline (5-phenyl-2-imino-4-oxo-oxazoleidine, PIO), the main constituent of Tradon[1] some side-effects of the substance on both animals[2] and plants[3] were noted. BRABEC and RÖPER[4] and RÖPER[5] observed influences of pemoline on mitotic index and mitotic cycle. While studying the effects of pemoline, its influence on the action of colchicine and caffeine was investigated.

Materials and methods. Vicia faba seeds were germinated in moist sand, and after 5–6 days the main root was cut off up to 3 cm. The further culture was done in aerated

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distilled water and 10^{-6} g/ml or 3×10^{-4} g/ml pemoline solution[4], respectively, in permanent light at 25 ± 1 °C. The effects of colchicine and caffeine were studied under the same conditions.

Colchicine: The metaphase accumulation[6] in 3×10^{-4} g/ml pemoline and distilled water, respectively, was determined with help of 0.005% colchicine 0.5 up to 4 h, in addition to this the prophase frequencies.

Caffeine: The seedlings were transferred to 0.1% caffeine solution resp. 3×10^{-4} g/ml or 10^{-6} g/ml pemoline + 0.1% caffeine, and the changes of the mitotic index were studied.

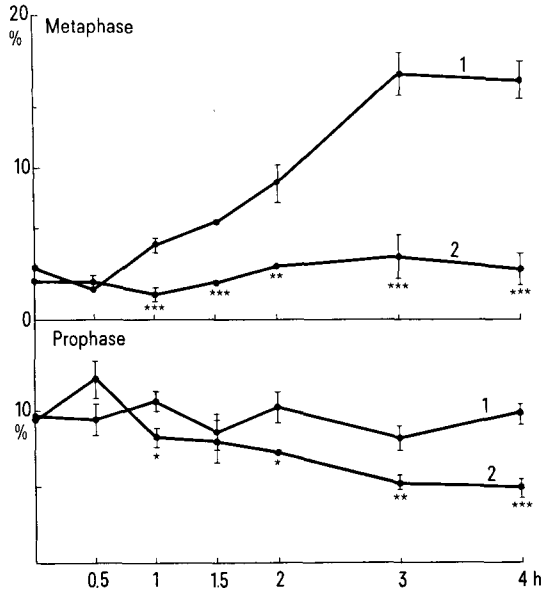
The determination of the mitotic index[7] and the phase frequencies was done at feulgen stained squash preparations; the results of 5 roots were used to calculate the mean and standard deviation.

Results. 0.005% colchicine: The Figure shows a slightly decreasing metaphase index in the control during the first $^1/_2$ h, while the prophase frequency is unchanged; this means a slow-down of prophase → metaphase proliferation or a stimulated metaphase → anaphase proliferation. Then the metaphase index increases up to 3 h. The prophase frequency is not significantly alternating (F 1.0), so there are no pronounced aberrations of the interphase → mitosis proliferation. Anaphases can be found up to 1 h after beginning of the treatment, but they are irritated soon. In 3×10^{-4} g/ml pemoline there is no metaphase accumulation, and the prophase frequency is significantly decreasing after a short increase (F 8.7), a consequence of a delayed G 2 → prophase proliferation. The reduced effect of colchicine is manifested in the frequencies of the anaphases, that are to be found up to 4 h only slightly irritated. C – metaphases and C-telophases were observed not before 3 h treatment. In stronger colchicine concentrations 3×10^{-4} g/ml pemoline have no marked effects on metaphase accumulation[5].

Table I. Mitotic index (%) during treatment with 0.1% caffeine + pemoline

Time (h)	Control	10^{-6} g/ml	3×10^{-4} g/ml	F
0	14.84 ± 1.48[a]	17.26 ± 0.67	13.97 ± 1.97	1.34 n.s.
0.5	9.80 ± 1.60	17.75 ± 0.37	12.48 ± 0.80	14.69[b]
1	7.25 ± 0.43	16.45 ± 1.07	9.62 ± 0.99	29.64[b]
1.5	5.53 ± 1.39	14.36 ± 1.61	10.81 ± 0.69	11.83[c]
9	0.38	14.94 ± 1.11	13.76 ± 2.24	31.28[b]
12	0.00	14.85 ± 1.56	17.49 ± 2.32	
24	—	3.02 ± 1.00	2.90 ± 0.98	

[a] Standard error of the mean. [b] $p < 0.1\%$; [c] $p < 1\%$.



Metaphase frequency (above) and prophase frequency (below) during treatment with 0.005% colchicine. Abscissa: time of treatment. 1: control, 2: 3×10^{-4} g/ml pemoline. The standard error of the mean is indicated as well as the degree of the significance in the *t*-test: Without marking: not significant, * $p < 5\%$; ** $p < 1\%$; *** $p < 0.1\%$.

Caffeine: Table I shows a strong decrease of mitotic index in the control up to 12 h, while it is unchanged in 10^{-6} and 3×10^{-4} g/ml pemoline; after further 12 h treatment, the damages are so strong that the mitotic index decreases, too. There were highly significant differences in the mitotic indices between controls and pemoline treatment. In addition to the action on mitotic index, pemoline showed further positive effects: the known aberrations caused by caffeine[8] could be observed only to a certain degree during pemoline treatment. Although there are no marked differences of the mitotic indices up to 12 h treatment with pemoline + caffeine, marked influences on phase frequencies can be stated (Table II): In all concentrations there is an early decrease of metaphases, even when the mitotic index is increasing; the metaphases do not reach the former values. On the other hand, the anaphases increase during pemoline + caffeine treatment, have a minimum after 1 or 1.5 h, and increase again. The prophases show a behaviour similar to that of mitotic index, while telophases behave like anaphases. Binucleate cells were watched during all treatments, i.e. it seems to be that pemoline has no influence on the action of caffeine on cytokinesis. The constant mitotic index during simultaneous treatment with pemoline shows that pemoline reduces the influence of caffeine on interphase and the G 2 → prophase proliferation; when treated with 3×10^{-4} g/ml pemoline, the prophase frequency at 9 and 12 h is even increased.

Discussion. The results show that pemoline is able to reduce and compensate the action of 2 drugs that have similar effects. This suggests similar sites of action, too. Since pemoline has an influence on mitotic time and the time of interphase[5], a binding to the mitotic apparatus similar to that of colchicine[9] seems possible, as well as an effect on replication and transscription. The positive effects of pemoline on DNA-repair after irridiation with X-rays[2] could explain the compensation of the action of caffeine, that is not restricted to mitosis itself. As typical aberrations induced by caffeine[8] are reduced, the toxic effects are stopped for some time. Pemoline seems to compensate the action of caffeine not entirely, because there remain influences on phase frequency and cytokinesis. In contrast to this the antagonism to colchicine

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Table II. Mitotic index (MI) and phase frequencies

Time (h)	Control					10^{-6} g/ml					3×10^{-4} g/ml				
	MI	P	M	A	T	MI	P	M	A	T	MI	P	M	A	T
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
0.5	66.0	70.3	46.9	57.9	83.3	102.8	112.6	45.3	137.4	119.4	89.3	99.9	37.8	127.7	138.6
1	48.9	53.2	41.9	30.6	58.1	95.3	103.4	80.9	104.9	70.4	68.9	82.2	48.0	61.2	69.3
1.5	37.3	40.6	37.9	24.6	33.9	83.2	93.5	79.7	61.8	49.5	77.4	93.5	44.4	57.7	115.0
9						86.6	82.4	71.6	119.5	110.2	98.5	127.0	46.2	93.5	108.7
12						86.0	84.6	54.1	150.4	104.2	125.2	159.8	74.0	97.7	127.6

P, prophase; M, metaphase; A, anaphase; T, telophase; corresponding to the values at $t = 0$ (= 100%) during treatment with 0.1% caffeine and pemoline.

for some time nearly is total. Since gibberellic acid[10] as well as IAA[11] show antagonistic effects to colchicine, too, and have an influence on the mitotic index[10,11] similar to that of pemoline[4], the effect of pemoline on mitosis can be compared with that of growth factors.

Summary. Pemoline, the constituent of Tradon, is able to slow down the decrease of the mitotic index caused by 0.1% caffeine in roots of *Vicia faba*, and mitotic aberrations are reduced. With 0.005% colchicine and 3×10^{-4} g/ml pemoline, no metaphase-accumulation can be observed, and anaphase-disorder is delayed.

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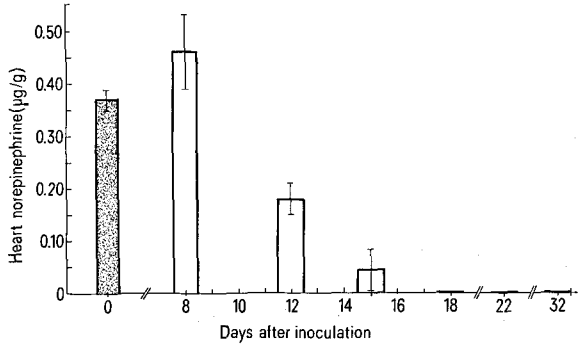
Depletion of Heart Norepinephrine in Experimental Acute Myocarditis Caused by *Trypanosoma cruzi*

Trypanosoma cruzi Chagas, 1909 is the causative agent of South American trypanosomiasis or Chagas', disease, one of the most frequent and dangerous illnesses of South America, with an estimated minimum of 7 million infected individuals[1]. Although parasitism attacks practically any organ, cardiac involvement is the most important clinical feature of Chagas' disease accounting for 87% of the deaths for which the disease is responsible[1]. It is now well established that in the course of *T. cruzi* infection there are lesions of the autonomic nervous system[2,3] resulting in extensive destruction of parasympathetic ganglion cells in human[4] and experimental[5] material. These lesions have been claimed[1] to be responsible for all the late manifestations of Chagas' disease, represented chiefly by anatomical and functional disorders of hollow muscular organs such as heart, esophagus and colon. Although the evidence for involvement of the parasympathetic nervous system is now generally accepted, the damage caused by *T. cruzi* on the sympathetic nervous system is a matter of controversy[6]. We have investigated this problem by estimating the sympathetic neurotransmitter norepinephrine (NE) in the heart of rats inoculated with *T. cruzi*.



The time course of norepinephrine depletion of the heart in rats inoculated with *Trypanosoma cruzi*. The mean values and SEM (indicated by bars) for heart NE concentrations are plotted for normal rats (12 animals) and at 8 (3 animals), 12 (3 animals), 15 (4 animals), 18 (4 animals), 22 (2 animals) and 32 days (3 animals) after inoculation.

Material and methods. Male and female Holtzman rats aged 27–30 days, and weighing 45–65 g were inoculated i.p. with blood containing 300,000 trypomastigotes of the Y-strain. Under these conditions the mortality was only 15%. At different periods after inoculation, the rats were sacrificed under ether anesthesia and their hearts were washed clean of blood by a quick saline perfusion, removed, blotted, weighed, immersed in 0.8 *N* perchloric

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