periments, were lightly anesthetized with ether and injected into an ear vein, randomly, with 1.0 ml of either apyrogenic saline or leukocytic pyrogen (LP)[11]. Upon regaining consciousness (usually in less than 10 min), the animals were placed into the cool end of the thermocline (again end-to-end gradient from 27 to 38 °C) and allowed to seek their preferred temperature for the next 4 h. The results are illustrated in figure 1. The T_{bo}s of the saline-injected guinea-pigs stabilized at 37.8 ± 0.4 °C after 40 min. By contrast, those of the LP-treated animals rose rapidly for the first 70 min, and then very slowly during the following 50 min, essentially stabilizing. After 120 min, they gradually declined. Defervescence was completed in 240 min post-LP injection. Over the 4 h of their exposure to the thermocline, the saline-treated animals gradually moved toward warmer T_as, from 28.7 ± 0.5 to 29.7 ± 0.4 °C. By contrast, the LP-treated guinea-pigs, coincidentally with the rising of their fever, sought a significantly warmer environment than the control animals during the first 70 min. During the following 50 min, i.e., during the period of high stable T_{bo}, they selected progressively lower T_as. During defervescence, they moved more rapidly to even cooler temperatures, and by the end of the experiment, they were at T_as not different from those of the controls.

Beginning 2 days after these experiments, each of the above animals was anesthetized with sodium pentobarbital and guide cannulas were implanted under stereotaxic guidance bilaterally into the preoptic area; the position of the cannula tips was verified histologically at the conclusion of these experiments[10]. 4 days after this surgery, each animal again was placed into the cool end of the thermocline (end-to-end gradient as before from 27 to 38 °C) to run freely for 2 h; no injections were given. Under these conditions, the animals selected 29.1 ± 0.4 °C after 40 min of random activity; T_{bo} averaged 38.0 ± 0.2 °C.

On subsequent days, 1.0 µl of either apyrogenic saline or LP, in separate randomized experiments, was injected bilaterally into the preoptic area of each guinea-pig, without anesthesia. The animal was then immediately placed into the cool end of the thermocline (gradient as before), and allowed to seek its preferred temperature for the next 4 h. T_{bo}, T_a, and animal location were monitored as before. The results are shown in figure 2. While the T_{bo}s of the saline-treated guinea-pigs remained stable at 37.8 ± 0.5 °C throughout the 240-min exposure, those of the LP-treated animals increased immediately, reaching their fastigium at 100 min. Defervescence began at 110 min and was completed by 240 min. The control guinea-pigs remained in 29.0 ± 0.4 °C throughout their exposure to the thermocline; by contrast, from 50 min post-LP onward, the LP-treated animals chose increasingly warmer T_as, coincidentally with the rising phase of their fever. They selected their highest T_a at 100 min, then gradually moved again toward cooler temperatures.

Hence, the present results would permit the conclusion that the preoptic area integrates not only the autonomic, as shown earlier[10], but also the behavioral components of fever in adult guinea-pigs.

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The mechanical and biochemical effects of pentoxifylline on the perfused rat heart[1]

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Summary. Perfusion of the isolated rat heart at constant heart rate and coronary flow with the inhibitor of cyclic nucleotide phosphodiesterase, pentoxifylline (10^{-4} moles/l), produced no significant effect on the maximum rate and the peak of contraction, but increased the maximum rate of relaxation. cAMP level and cAMP-dependent protein kinase activity were increased in the absence of changes in cGMP. The results were identical in hearts of reserpinized rats.

Increases in cardiac contractility are associated with increased intracellular levels of adenosine 3′:5′ cyclic monophosphate (cAMP)[3]. Catecholamines that stimulate myocardial β-adrenergic receptors produce their positive inotropic response by increasing cAMP production[4]. Myocardial cAMP is also increased by inhibition of its breakdown to 5′ AMP by phosphodiesterase inhibition[5–7]. The increase in intracellular concentration of cAMP is thought to activate a cAMP-dependent protein kinase which in turn phosphorylates specific cellular proteins, such as proteins of myocardial sarcolemma[8], the contractile protein troponin[9] and the phospholamban in the sarcoplasmic reticulum[10]. This protein phosphorylation might lead to changes in contractility and relaxation through alteration of Ca^{++} movement. Protein kinase-dependent phosphorylation of phospholamban should result in an enhancement of Ca^{++} transport into the sarcoplasmic reticulum[11] causing accelerated relaxation[12]. An accelerated relaxation has been detected in our laboratory with isoproterenol and was mimicked by dibutyryl 3′:5′-cAMP[13].

The possibility that guanosine 3′:5′ cyclic monophosphate (cGMP) may have regulatory actions on the heart antagonistic to those of cAMP has been reported[14]. Cholinergic agents have been shown to increase myocardial cGMP level and to decrease contractile force[15].

The reported inotropic and relaxant effects of phosphodiesterase inhibitors are controversial[3,6,16–20]. The present study was undertaken to provide information about the mechanical behavior of the perfused rat heart under the effect of the phosphodiesterase inhibitor, pentoxifylline, and its relation with myocardial cAMP-dependent protein kinase activity, cAMP and cGMP levels.

Methods. Heart perfusion: Langendorff's technique of perfusion was employed using isolated hearts from white rats weighing 250-350 g as described in a previous paper[13]. The main conditions characterizing this preparation were: constant coronary flow (9 ml/min), perfusion pressure between 50 and 90 mm Hg, temperature of the perfusion medium 37 °C. The hearts were paced at a constant rate of 200 beats/min with an intensity slightly higher than threshold. To prevent interference from atrial beats, total heart block was performed by crushing the atrial septum. Composition of the Ringer solution in mmoles/l was: NaCl 128.3; KCl 4.7; $CaCl_2$ 1.35; $NaHCO_3$ 20.0; NaH_2PO_4 0.4; $MgCl_2$ 1.1 and glucose 11.1. The hearts were perfused for 30 min with normal Ringer solution for stabilization and then for a 15-min period either with the same solution (control group) or with Ringer containing pentoxifylline 10^{-4} M. This concentration was chosen from preliminary trials which showed an increase of cAMP-dependent protein activity. At the end of the perfusion period, hearts were quickly frozen with Wollenberger clamps previously cooled in liquid nitrogen. The powdered tissue was stored at −70 °C until assayed.

In experiments with depleted hearts, the animals were injected with reserpine (5 mg/kg) 24 h before the experiment. Noradrenaline was depleted by 92± 12% as determined by the method of Häggendal[21].

Developed tension and its first derivate obtained by electronic differentiation were measured in a segment of the left ventricle wall by sewing a rat strain gauge arch (J.C. Warren, Charleston, S.C. USA). Developed tension (T), maximal rate of tension (+Ṫ) and maximal rate of relaxation (−Ṫ) were measured at a paper speed of 50 or 100 mm/sec. The values were expressed as the difference in percent of the value at zero time after the stabilization period.

Relative effects on maximal velocity of contraction as compared with those on maximal velocity of relaxation were assessed by the ratio +Ṫ/−Ṫ.

Protein kinase assay: cAMP-dependent protein kinase assay was carried out in approximately 50 mg of powdered tissue which was suspended at 4 °C in 0.75 ml of 10 mM potassium phosphate buffer containing 10 mM EDTA and 0.5 mM theophylline at pH 6.8 and homogenized with 3 up-and-down strokes of a motor-driven Teflon pestle. The homogenate was immediately centrifuged at 12,000× g for 20 min at 4 °C. The protein kinase activity in the supernatant fraction was determined by measuring the phosphorylation of histone from (γ^{32}P) ATP in the presence and absence of added 2 µM cAMP. The reaction was started by adding 20 µl of the supernatant fraction to 50 µl of solution containing 17 mM potassium phosphate (pH 6.8), 0.33 mM (γ^{32}P) ATP (about 35 cpm/pmole), 6 mM magnesium acetate, 0.5 mg of histone and 2 µM cAMP where indicated. The incubation was carried out at 30 °C for 5 min and stopped by pipetting a 50-µl aliquot of the reaction mixture onto a 1×2 cm filter paper disc which was immediately dropped into ice cold trichloroacetic acid (TCA) (10 ml/disc). The filter paper was washed according to the method of Wastila et al.[22] and counted in 10 ml of toluene cellosolve scintillation fluid. cAMP-dependent protein kinase activity is expressed as the protein kinase activity ratio i.e. the ratio of activity in the absence of cAMP to the activity in the presence of 2 µM cAMP to stimulate the kinase (−cAMP/+cAMP) maximally[23].

Cyclic AMP and cyclic GMP assay: Cyclic AMP and cyclic GMP were estimated by the radioimmuno assay of Steiner et al.[24], with acetylation of the samples as supplied by New England Nuclear. To measure cAMP approximately 10 mg of frozen heart tissue samples were homogenized in 6% TCA at 4 °C. To determine the recovery of cAMP after purification, approximately 4000 cpm ^3H_c AMP were added to the TCA extract. The deproteinized homogenate was centrifuged at 2500× g at 4 °C for 15 min and the supernatant was extracted 4 times with 5 volumes of water-saturated diethylether. The ether phase was discarded and the samples placed in a water bath at 70-80 °C to evaporate to dryness under a stream of air. The same procedure was followed to measure cGMP using about 50 mg of frozen heart tissue samples with ^3H_c GMP (1500 cpm) as a tracer. The specificity of this method allows the determination of cAMP and cGMP without chromatography of the tissue extract[25-27], since minimal crossreactivity has been reported even with 1000-fold increase in cAMP relative to cGMP[26].

Cyclic nucleotides and protein kinase activity ratio values were statistically analyzed with Student's t-test for independent samples. The statistical significance for the mechanical parameters were obtained by Student's t-test for paired samples. Differences were considered statistically significant when p-values were < 0.05.

Results. 17 control experiments with 15 min of perfusion under control conditions were performed. No significant changes were observed in the mechanical parameters during and after the 30 min of the stabilization period. The values for the mechanical parameters after 15 min of

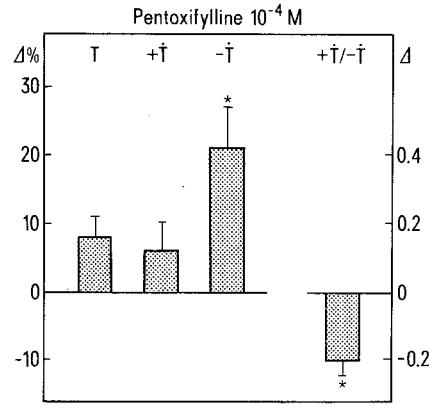

Fig. 1. Effects of 15 min of perfusion with pentoxifylline 10^{-4} M on developed tension (T), maximal velocity of tension development (+Ṫ), maximal velocity of relaxation (−Ṫ) and the ratio between maximal velocities of contraction and relaxation (+Ṫ/−Ṫ). Data are means ± SE expressed as differences (+Ṫ/−Ṫ) or percentual differences (T, +Ṫ, −Ṫ) from the 30th min of the stabilization period. Asterisks indicate statistically significant differences, $p < 0.05$.

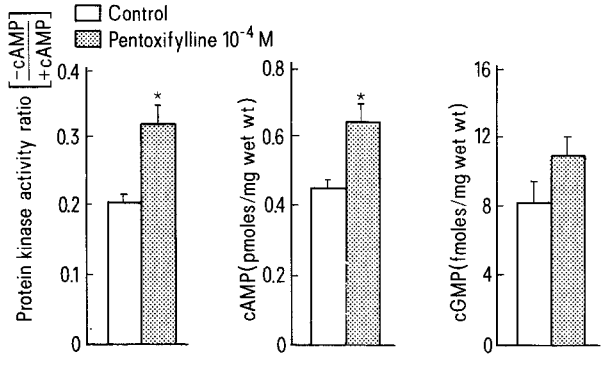

Fig. 2. Biochemical changes in hearts perfused during 15 min with and without pentoxifylline 10^{-4} M. cAMP levels and cAMP-dependent protein kinase activity ratio rose significantly. The cGMP increase was not statistically significant. Data are means ± SE. Asterisks indicate statistically significant differences from control values with $p < 0.05$.

perfusion were: T ($-0.20\pm2\%$); $+\dot{T}$ ($1\pm2\%$) and $-\dot{T}$ ($6\pm3\%$).

The effect of pentoxifylline on myocardial performance is shown in figure 1. In 9 experiments after 15 min of infusion the only statistically significant changes in mechanical parameters were an increase of $-\dot{T}$ by $21\pm6\%$ and accordingly a decrease of the $+\dot{T}/-\dot{T}$ quotient by 0.20 ± 0.05. In experiments on reserpine pretreated rats, essentially the same response to pentoxifylline was found with a significant increase in $-\dot{T}$ ($15\pm3\%$) and decrease in $+\dot{T}/-\dot{T}$ of (0.13 ± 0.06).

Pentoxifylline infusion increased significantly the level of cAMP and the protein kinase activity ratio (figure 2).

No significant increase of the cGMP level was observed at the end of pentoxifylline infusion.

Discussion. In early studies on the effect of epinephrine in the intact heart it was shown that the observed increase in contractility was accompanied by an increase in the rate of relaxation[28]. This effect was confirmed later with isoproterenol in cat papillary muscles[18,29] and in the perfused rat heart[13]. The effect of β agonists on the relaxation phase was suggested to be mediated through an increase in cAMP since an increased calcium uptake by the sarcoplasmic reticulum was found which was related to an increase in the rate of relaxation following the addition of cAMP[30]. Moreover, in experiments from our laboratory in which the ratio of maximal velocities of contraction to relaxation ($+\dot{T}/-\dot{T}$) was used as an index of relaxation, it was shown that the effect of isoproterenol on relaxation was mimicked by dibutyryl cAMP[13].

The present results show a statistically significant increase in the rate of relaxation ($-\dot{T}$) after 15 min of pentoxifylline infusion with a corresponding decrease in the ratio value ($+\dot{T}/\dot{T}$). This effect can not be attributed to endogenous release of catecholamines since the experiments with hearts depleted of catecholamines by reserpine gave identical results.

Along with the increase in relaxation pentoxifylline increased cAMP and cAMP-dependent protein kinase activity ratio with an increase in cGMP levels which do not reach a statistically significant level.

The accelerating effect of pentoxifylline on relaxation in the perfused rat heart under our experimental conditions can be related to activation of the protein kinase system by an increase of the intracellular cAMP.

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The involvement of porphyrogenic steroids in the development of experimental porphyria[1]

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Summary. Hexachlorobenzene alters the hepatic steroid metabolism, and it was suggested that a porphyria was induced by overproduction of 5β-H-steroids. Structurally similar chlorinated hydrocarbons (pentachlorobenzene, pentachlorophenol, 2,4,5-trichlorophenol) without porphyrogenic activity did not affect the steroid metabolism in rat liver.

A great number of substances are able to provoke a hepatic porphyria in men and laboratory animals[2]. Furthermore, it was found that the ratio of 5β/5α-steroids in urine was elevated in man after administration of the porphyrogenic drug phenobarbital[3]. It was concluded that the activity of the hepatic NADPH: Δ^4-3-oxosteroid-5α-reductase was inhibited or diminished following administration of this drug. The decrease of NADPH: Δ^4-3-oxosteroid-5α-reductase ac-