1,4-Dichlorobenzene had a low potential for causing porphyria (table 2). After 30 and 60 days there was a dose-dependent increase in the liver weight which is in agreement with previous results[17]. There were only slight increases in liver porphyrins even after 120 days. There were no differences between the treated animals and controls in the urinary excretion of ALA, PBG or porphyrins.

1,2,4-Trichlorobenzene also demonstrated a lack of ability to cause porphyria although the liver weight did increase (table 3). The increases seen in liver porphyrins were very small compared to those observed with hexachlorobenzene. Urinary excretion of ALA and PBG were not elevated at any time at any dose. Porphyrin excretion was only minimally increased at 200 mg/kg after 30 and 90 days.

The results, while confirming the ability of hexachlorobenzene to produce severe hepatic porphyria, indicate that 1,4-dichlorobenzene and 1,2,4-trichlorobenzene do not share this property. Even at doses up to 200 mg/kg for 120 days, there were no changes which would indicate a potential problem involved with the intake of these chemicals.

17 G. P. Carlson and R. G. Tardiff, Toxic. appl. Pharmac. *36*, 383 (1976).

The opposed influences of β-adrenergic stimulation and adenosine on the frequency-force relationship of isolated left atria of guinea-pigs

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Summary. The normal frequency-force relationship of left guinea-pig atria can be largely suspended when strong β-adrenergic stimulation by orciprenaline is antagonized by the negative inotropic effect of adenosine, so that contraction amplitude is nearly equal at an intermediate level over a wide range of stimulation rates. Curves obtained with a new method for recording continuous frequency-force loops are presented.

The dependence of myocardial contractility on heart rate or stimulus pattern, known as interval-strength relationship[1], certainly reflects basic processes of excitation-contraction coupling[2]. Accordingly, this relationship has been shown to be characteristically influenced by different types of intropic interventions, and further investigations should help to clarify open questions of both excitation-contraction coupling and inotropic mechanisms[1,2].

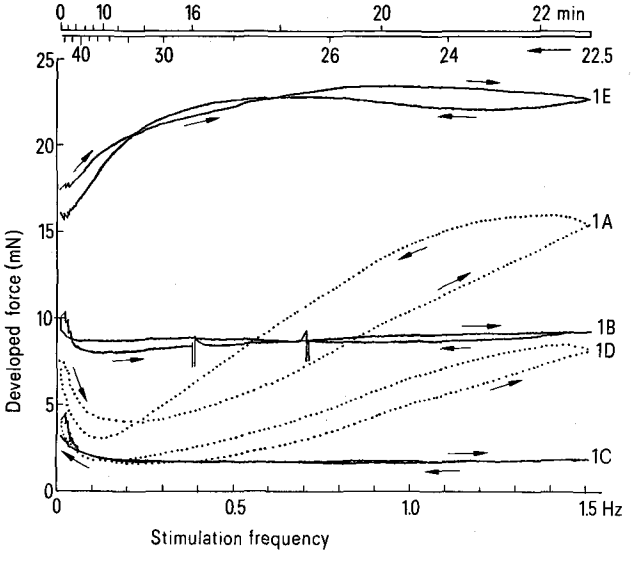

Fig. 1. 5 continuous frequency-force loops on the left atrium of a 190 g guinea-pig. In 45 min the frequency is changed from 0.007 Hz to 1.5 Hz and back to 0.007 Hz (time scale above the panel). Resting force 7.5 mN; 32 °C; 2.5 mM calcium. Curves 1B, 1C and 1E are original registrations, 1A and 1D were replotted. The arrows indicate the direction of the automatic frequency change. *1A* Initial control. *1B* With 10^{-5} M orciprenaline[8] plus 3×10^{-4} M adenosine; 2 extrasystoles. *1C* With 10^{-5} M orciprenaline, adenosine increased to 10^{-3} M. *1D* Control curve after thorough wash-out of drugs. *1E* With 10^{-5} M orciprenaline alone.

Methods. The various approaches to conduct interval-strength studies[2] all seem quite tedious, e.g. repeated determination of steady-state contractile strength at a number of selected stimulation rates, from which 'frequency-force curves' can be plotted. In order to facilitate and standardize this procedure, we use a new stimulator[3] which can vary the driving frequency ν continuously as exponential function of time ($\nu \sim e^{t/k}$ and $\nu \sim e^{-t/k}$); an upper frequency limit is set for automatic reversal from rise to fall, usually at 1.5 Hz. The time constant k is set long enough (about 4 min) to allow almost complete cumulation of inotropic effects of activation[1] in the preparation. Peak developed isometric force is electronically processed for continuous registration[4]: 1. With an x–y recorder continuous frequency-force plots are obtained by directly recording the developed force on the y-axis against the changing frequency on the x-axis (figures 1 and 2, 1A–E, 2A–E). 2. Force responses to step changes of frequency are recorded on a conventional slow recorder (figure 2b, c, e).

All experiments were performed on isolated left atria of young male guinea-pigs. Krebs-Henseleit solution[5] with 15 mM glucose was rapidly recirculated past the tissue by bubbling with 5% CO_2 in O_2 in a special bath vessel[6,7]. The atria were stimulated slightly above threshold at 0.1–0.2 msec via a punctate cathode[6].

Results and discussion. On 150 preparations more than 1000 curves were determined under a variety of experimental conditions. The interaction of β-adrenergic

1 J. R. Blinks and J. Koch-Weser, J. Pharmac. expl Ther. *134*, 373 (1961).
2 J. Koch-Weser and J. R. Blinks, Pharmac. Rev. *15*, 601 (1963).
3 The new stimulator was designed and built by Rolf Englert, Electronics Development Laboratory of this institute.
4 M. Siess, H. J. Keller, E. Schare, J. Geissler and G. Müller, J. molec. cell. Cardiol. *1*, 261 (1970).
5 H. A. Krebs and K. Henseleit, Hoppe-Seylers Z. physiol. Chem. *210*, 33 (1932).
6 J. R. Blinks, J. appl. Physiol. *20*, 755 (1965).
7 H. J. Mensing, G. Jung and K. Stieler, Naunyn-Schmiedebergs Arch. Pharmak. *293*, R70 (1976).

stimulation by orciprenaline[8,9] and the negative inotropic effect of adenosine on guinea-pig atria[10] was found to be quite striking. In figure 1, curves from a single preparation are shown, representative for 15 similar experiments. 1A is a control frequency-force curve typical for mammalian atrium[2]. In 1B nearly maximal β-adrenergic stimulation by 10^{-5} M orciprenaline is antagonized by the simultaneous action of 3×10^{-4} M adenosine. With these opposed interventions contractile force is nearly independent of frequency above 0.1 Hz. This frequency-force loop (1B) cuts the rising control loop (1A) at an intermediate level, and more adenosine (10^{-3} M) displaces the curve downward in a roughly parallel manner (1C). Curve 1D, obtained after thorough wash-out of the drugs, shows about 50% of the initial force values (1A) at all frequencies, which is not unusual after 4.5 h. Finally, with 10^{-5} M orciprenaline alone, curve 1E levels off at a high developed force. Such a flattening of the frequency-force relationship is produced by many stimulatory agents if the maximal contraction force a preparation can generate is approached[2].

The strongly negative inotropic effect of 3×10^{-4} M adenosine alone is presented in figure 2 in an experiment on another preparation: Developed force is reduced by 80% at 1.5 Hz, but only slightly at the very lowest rates (compare 2A and 2B; representative for 10 experiments). The effects of the negative inotropic agents, carbachol[2] and verapamil (or D 600[11]) alone are very similar to those of adenosine, but no combination of verapamil or carbachol with orciprenaline produced such a flat frequency-force curve as adenosine plus orciprenaline (unpublished results). Nor was it found when adenosine was combined with the positive inotropic agent, ouabain, or as in curve 2C, with high extracellular calcium (final concentration 8 mM; 3×10^{-4} M adenosine). This loop cuts the rising control loop 2B, as do loops 2D, E with orciprenaline (10^{-5} M) and adenosine (3×10^{-4} M, 10^{-4} M, respectively). However, in loop 2C with high calcium plus adenosine some rise of contraction force is seen above 0.5 Hz, and at low frequencies contraction amplitude is considerably enhanced. Thus, the nearly frequency-independent inotropic state found for the interaction of orciprenaline with adenosine may be quite unique. To our knowledge, this is the first report of conditions under which contraction force is essentially equal at an intermediate level over a large frequency range.

The differences described above are also clearly reflected in contractile responses to step changes of frequency. After a step increase from rest to 1.5 Hz under control conditions, developed force decreases quickly, followed by a slow rise over 3 min to steady state level (2b). With adenosine antagonized by increased calcium as in 2C, this slow phase does not appear to be principally altered (2c); however, with the orciprenaline-adenosine combination as in 2E, this slow phase is replaced by 3 low amplitude phases (2e), suggesting the differentiation of more than just 1 negative and 1 positive inotropic effect of activation[2,12].

The hypothesis that cAMP mediates β-adrenergic effects in the heart[13] has gained considerable support[14]. According to the analysis of Koch-Weser and Blinks, sympathomimetic amines augment contractile strength mainly by enhancing the intrinsic mechanism responsible for the rise in contractility with increasing frequency[2]. Therefore, Robison et al. suggested in 1965 the involvement of a cAMP-regulated enzyme or enzyme system in this mechanism[13]; a protein kinase might be proposed today[15–18]. The mode of action of adenosine has received growing interest, with emphasis on a possible influence on enzymes of the cAMP system[19]; inhibition of adenylate cyclase[20] and cAMP-regulated membrane-bound protein kinase[21,22] has been described. If these sites of action are correct, the nearly frequency-independent inotropic state presented could result from manipulations of the cyclic nucleotide system. It would seem worthwhile to consider whether changes of membrane protein phosphorylation may be involved in the mechanisms underlying the normal interval-strength relationship.

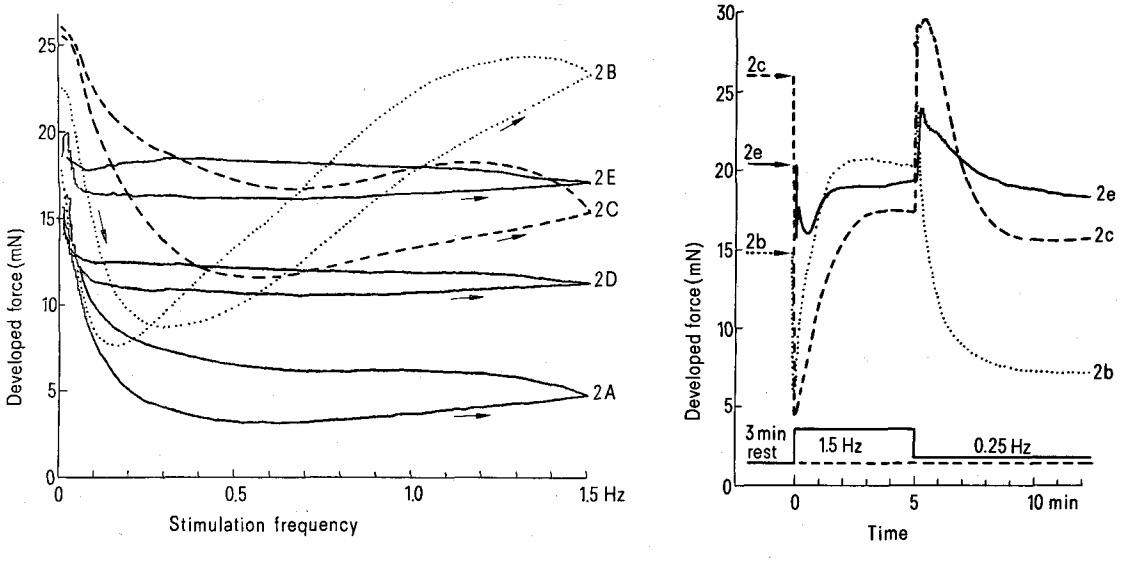

Fig. 2. All curves from a single left atrium of a 210 g guinea-pig. Same experimental conditions as in figure 1. 2A, 2D, 2E and 2e are original curves, 2B, 2C, 2b and 2c are retracted. Left panel: 5 frequency-force loops. *2A* With 3×10^{-4} M adenosine. *2B* Control. *2C* 3×10^{-4} M adenosine with 8 mM calcium (final concentration). *2D* 3×10^{-4} M adenosine plus 10^{-5} M orciprenaline. *2E* 10^{-4} M adenosine plus 10^{-5} M orciprenaline. Right panel: Force responses to step changes of frequency as indicated under the tracings. *2b* Control. *2c* 3×10^{-4} M adenosine plus 8 mM calcium (final concentration). *2e* 10^{-4} M adenosine plus 10^{-5} M orciprenaline. The arrows show contraction amplitude after 3-min rest periods.

8 Alupent® Amp., Boehringer Ingelheim, West Germany.
9 S. R. O'Donnell and J. C. Wanstall, Br. J. Pharmac. *52*, 407 (1974).
10 T. De Gubareff and W. Sleator, Jr, J. Pharmac. expl Ther. *148*, 202 (1965).
11 D. Reinhardt, W. Roggenbach, U. Schmidt and H.-J. Schümann, Eur. J. Pharmac. *41*, 123 (1977).
12 V. Kruta and P. Braveny, Archs int. Physiol. Biochim. *69*, 645 (1961).
13 G. A. Robison, R. W. Butcher, I. Øye, H. E. Morgan and E. W. Sutherland, Molec. Pharmac. *1*, 168 (1965).
14 R. W. Tsien and R. Weingart, J. Physiol. *260*, 117 (1976).
15 G. A. Robison, R. W. Butcher and E. W. Sutherland, Cyclic AMP. New York, Academic Press 1971.
16 G. Brooker, Adv. cycl. Nucl. Res. *5*, 435 (1975).
17 J. A. Schneider and N. Sperelakis, J. molec. cell. Cardiol. *7*, 249 (1975).
18 H. Reuter and H. Scholz, J. Physiol. *264*, 49 (1977).
19 S. G. McKenzie, R. Frew and H.-P. Bär, Eur. J. Pharmac. *41*, 193 (1977).
20 H. P. Bär and S. G. McKenzie, in: Recent Advances in Studies on Cardiac Structure and Metabolism, vol. 3: Myocardiol Metabolism, p. 311. Ed. N. S. Dhalla. Baltimore, University Park Press 1973.
21 T. Ueda, H. Maeno and P. Greengard, J. biol. Chem. *248*, 8295 (1973).
22 E.-G. Krause, H. Will, B. Schirpke and A. Wollenberger, Adv. cycl. Nucl. Res. *5*, 473 (1975).

In vivo effects of *Escherichia coli* endotoxin on sulfobromophthalein clearance in the guinea-pig

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Summary. In vivo studies indicated that the primary effects of *E. coli* endotoxin on hepatic clearance of sulfobromophthalein were at the excretory level. Newborns were more sensitive to the LPS than older animals.

Cholestatic jaundice associated with gram-negative bacterial infections has been reported in humans, especially neonates[3-6], but the pathogenesis of the clinical entity is uncertain. Many of the effects of these bacteria have been attributed to their lipopolysaccharides (LPS)[7]. Recently, we reported that a purified LPS from *E. coli* reduced bile flow and sulfobromophthalein (BSP) excretion and increased BSP storage in the isolated perfused rat liver (IPRL)[8,9]. In addition, endotoxins from various species of bacteria have been described to inhibit the clearance of BSP from the blood of rats, rabbits, baboons and humans[10-13]. Since the incidence of cholestatic jaundice appears to be more prevalent in newborns than adult humans[3-6], it seemed of interest to compare the effects of an endotoxin on the excretory processes of animals of different ages. Guinea-pigs were chosen for the study as they are relatively large at birth and are sensitive to LPS[14]. Furthermore, the hepatic mechanisms for the clearance of BSP in these animals have been extensively studied[15,16] and are mature at 7 days[15].

Materials and methods. Hartley guinea-pigs (both sexes) at 1, 3 and 7 days were utilized in these studies. LPS from *E. coli* (055:B5) was purchased from Difco Laboratories. It was dissolved in sterile pyrogen-free saline just prior to use and administered (35 µg/100 g b.wt, i.p.; saline only in controls) between 9.30 and 10.30 h. 2 h later, BSP (1.2 mg/100 g[15]) was given by intracardiac injection. To

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3 J. Bernstein and A. K. Brown, Pediatrics *29*, 875 (1962).
4 J. R. Hamilton and A. Sass-Kortsak, J. Pediat. *63*, 121 (1963).
5 S. H. Ng and J. R. Rawstron, Archs Dis. Childh. *46*, 173 (1971).
6 D. F. Eley, T. Hargreaves and H. P. Lamberth, Br. Med. J. *2*, 75 (1965).
7 S. Kadis, G. Weinbaum and S. J. Ajl (ed.), Microbial Toxins, vol. 5, Bacterial Endotoxins. Academic Press, New York 1971.
8 R. Utili, C. O. Abernathy and H. J. Zimmerman, Gastroenterology *70*, 248 (1976).
9 R. Utili, C. O. Abernathy and H. J. Zimmerman, J. Lab. clin. Med. *89*, 471 (1977).
10 N. R. DiLuzio, R. A. Trejo and C. G. Crafton, J. reticuloendoth. Soc. *11*, 637 (1972).
11 R. L. Hirsch, D. G. McKay, R. I. Travers and R. K. Skraly, J. Lipid Res. *5*, 563 (1964).
12 K. Holper, R. A. Trejo, L. Brettschneider and N. R. DiLuzio, Surg. Gynec. Obstet. *136*, 593 (1973).
13 T. F. Blaschke, R. J. Elin, P. D. Berk, C. S. Song and S. M. Wolff, Ann. intern. Med. *78*, 221 (1973).
14 J. W. Uhr, J. expl Med. *115*, 685 (1962).
15 J. Goldstein, S. Schenker and B. Combes, Am. J. Physiol. *208*, 573 (1965).
16 S. Schenker, J. Goldstein and B. Combes, Am. J. Physiol. *208*, 563 (1965).

Effect of *E. coli* endotoxin (LPS) on sulfobromophthalein (BSP) retention, storage in the liver and release of aspartate aminotransferase (GOT)*

Age in days	Treatment	Percent BSP retention at 30 min	BSP concentration in liver µg/g liver	BSP in liver as percent of applied dose	Serum GOT (IU/l)
1	None (6)	1.94 ± 0.29	67 ± 20	21 ± 6	48 ± 9
1	LPS (8)	6.39 ± 0.83***	136 ± 20**	52 ± 5***	82 ± 10**
3	None (6)	2.85 ± 0.58	13 ± 3	4 ± 1 (3)	60 ± 9
3	LPS (7)	6.58 ± 1.00***	95 ± 15*** (6)	31 ± 5*** (6)	82 ± 11 (4)
7	None (7)	1.61 ± 0.36	14 ± 5	4 ± 5	45 ± 3
7	LPS (8)	2.17 ± 0.39	35 ± 6**	11 ± 6**	66 ± 11 (4)

* All data are expressed as means ± SE. The number in parentheses are the number of experiments in each group. ** $p < 0.05$. *** $p < 0.01$.