bring about the response. The degree of stimulatory effects of alloimmune sera depended on serum concentrations and was directly proportional to the cytotoxic titers and the number of immunizations; this suggests that positive ino- and chronotropic effects of sera could depend or be related to the presence of antibodies.

The notion of adrenergic participation in the effects of alloimmune sera is strongly supported by the observation that after blockade of the beta adrenoceptors, all the stimulatory actions of antiserum were blunted.

In order to investigate if the beta adrenergic effect of the alloimmune sera was due to a direct post-synaptic reaction with the beta adrenoceptors or to an indirect action mediated by presynaptic release of endogenous norepinephrine, 6-OH dopaminized animals were used. After denervation, the positive inotropic and chronotropic action of alloimmune sera persisted, suggesting that the alloimmune sera could influence the post-synaptic sites on the plasma membrane.

The mechanism whereby alloimmune sera may trigger a beta adrenergic reaction is not known. Recently it was documented that antibodies raised against beta adrenergic receptors interact with a distinct site from the receptor for hormone interaction, but may still stimulate adenylate cyclase activity[13].

The present preliminary study indicates that alloimmune sera react efficiently with some unknown structure, somehow related to the beta adrenergic mechanism. This structure could be an antigenic determinant. Further studies will be required before any mechanism of action can be proposed.

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Positive inotropic and negative chronotropic effect of pantethine on isolated cardiac muscle of guinea-pigs

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Summary. It was demonstrated that pantethine, a component of co-enzyme A, produces positive inotropic and negative chronotropic actions in isolated cardiac muscle preparations from guinea-pigs.

A growth factor produced by *Lactobacillus bulgaricus* was discovered by Williams[2] and named LBF. LBF, later shown to be a substance that constitutes a part of the structure of co-enzyme A, is composed of pantothenic acid bound with β-mercaptoethylamine. LBF occurs in 2 forms: one being pantetheine and the other pantethine, its stable disulfide. These 2 forms convert into each other in vivo. The potential clinical usefulness of co-enzyme A, a substance essential to living organisms, has not been realized, owing to its in vivo degradation into pantethine and pantothenic acid. Pantethine, a co-enzyme A precursor, which is closer to co-enzyme A in structure than pantothenic acid, gives a high co-enzyme A yield in vivo (fig. 1). The present study was undertaken to determine whether chemically synthesized pantethine[3] has any effect on isolated atria and papillary muscle preparations from guinea-pigs.

Materials and methods. Male guinea-pigs weighing approximately 500 g were used. 1. Papillary muscle preparations obtained from the right ventricle using Gold and Cattell's method[4] were driven by electrical stimulation (rectangular wave, 5 msec duration, 1 Hz). These preparations were suspended in an organ bath containing Locke-Ringer's solution. The bath temperature was maintained at 37 °C and the solution was continuously aerated with a mixture of 95% O_2 and 5% CO_2. The composition of the Locke-Ringer's solution was as follows (mM): NaCl 154.0, KCl 5.63, $CaCl_2$ 2.16, glucose 11.0, $NaHCO_3$ buffer 2.4 (pH 7.4). The drug concentration given in this paper was the final concentration in the organ bath. Isometric tension of papillary muscle preparations was recorded by a strain gauge transducer. 2. Spontaneously beating atria were suspended in the same organ bath and the rate was recorded before and after administration of pantethine.

Results. 1. It was demonstrated that a pantethine concentration of 5×10^{-3} g/ml induced a significant increase in the isometric tension of the papillary muscles of guinea-pigs ($+17.6 \pm 8.8\%$, $p < 0.001$). Figure 2 shows the relationship between the concentration of pantethine and the contractile response of the papillary muscle preparations. No change in contractile force was observed at a concentration of 10^{-5} g/ml, but the contractile force increased dose-dependently at a concentration of 10^{-4} and 5×10^{-3} g/ml. The time-action curve for the positive inotropic effect of pantethine was also studied. Maximum tension was obtained approximately 10 min after administration of pantethine at a concentration of 5×10^{-3} g/ml. 2. The effect of pantethine on the heart rate in spontaneously beating atria of guinea-pigs was studied. Although pantethine concentrations of 10^{-5} g/ml did not produce any change in heart rate, concentration of 5×10^{-3} g/ml produced a significant

decrease in the rate of spontaneously beating atria ($-9.93 \pm 5.98\%$, $p < 0.02$ vs 10^{-5} g/ml) (fig. 3).

Discussion. Pantothenic acid is a required nutrient and it is believed that it has no outstanding pharmacological actions at ordinary doses. However, it has been demonstrated that exogenous pantethine produced an increase in intestinal motility in rabbits[5]. No reports are available concerning its action on the cardiac muscle. In this study, it was observed that at a concentration of 5×10^{-3} g/ml, pantethine produced a positive inotropic effect in the papillary muscle preparations of guinea-pigs. A negative chronotropic effect was also observed at the same doses in the guinea-pigs' atria. We have demonstrated that these positive inotropic and negative chronotropic actions are similar to those exerted by lower concentrations of ouabain[6]. However, ouabain was more than 10,000 times as effective as pantethine in positive inotropic and negative chronotropic actions (mol.wt of pantethine: 554.73, that of ouabain: 586.64).

Pantethine has a pharmacological effect in relatively high concentrations such as 10 mM. A specific study is necessary to determine its mode of action. For instance, it is not yet known whether pantethine has an effect on cell membrane permeability to calcium ions. With regard to pantethine distribution in rats, Tatezawa et al.[7] reported that the peak blood level of ^{14}C-labeled compounds was observed 8–16 h after the administration of a single oral dose of 200 mg/kg ^{14}C-labeled pantethine (pantethine-alanine-2-^{14}C). 7 days after administration of pantethine at a dose of 200 mg/kg/day, the radioactivity in the cardiac muscle of rats rose to 3.3 times that of the rats given a single oral dose. The biological half-life of ^{14}C-pantethine was 172.4 h. 8–16 h after a single oral administration of ^{14}C-labelled pantethine, radioactivity in various tissues including the myocardium was higher than that of the blood. However, no evidence was obtained about the rapidity of distribution of pantethine in cardiac muscle in particular. Although our study was not chronic, it is projected that in a chronic experiment, if the increased radioactivity of the myocardium of ^{14}C-pantethine-treated rats is associated with myocardial co-enzyme A concentration, the co-enzyme A biosynthesis induced by chronic pantethine administration may be responsible for the positive inotropic action of pantethine. The 10 mM of pantethine used to produce an inotropic action in Locke-Ringer's solution created no significant pH changes.

With regard to species, no reports are available in the literature concerning what different effects pantethine may produce on the myocardium of different species. One of the reasons that guinea-pigs are considered a good model for the examination of direct action on cardiac muscle may be that their sensitivity to cardiotonic drugs is higher than that of other species. In this experiment, pantethine produced a significant decrease in the heart rate of spontaneously beating guinea-pig atria. Pantethine's negative chronotropic effect has been observed in rats by Mochizuki et al. (unpublished data) and in dogs by Shibano and Abiko[8]. Mochizuki et al. studied the effect of 10^{-4} M pantethine on chronotropic action using the Langendorff preparation obtained from rats. Shibano and Abiko studied the effect of 40 mg/kg of pantethine i.v. on the heart rate of dogs. A species difference in respect to the negative chronotropic effect of pantethine was not observed in the present study. Pantethine has been shown to be converted to co-enzyme A

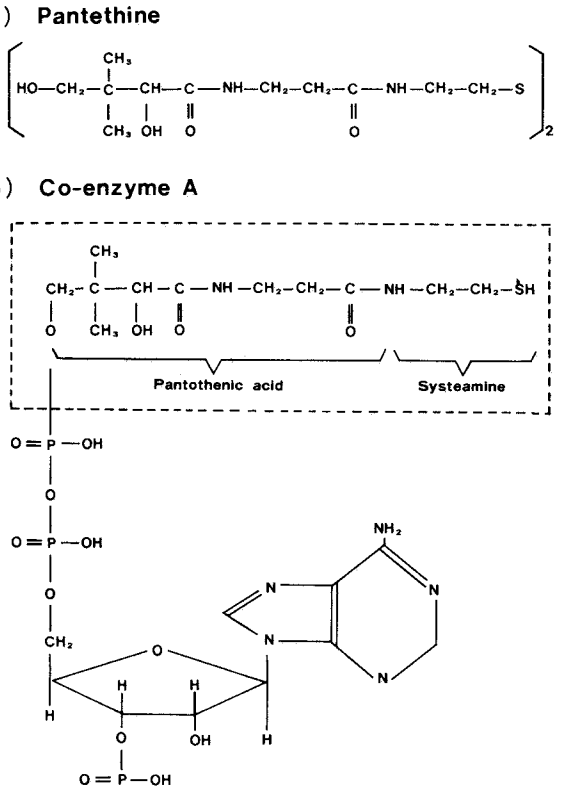

Figure 1. The chemical structures of pantethine and co-enzyme A.

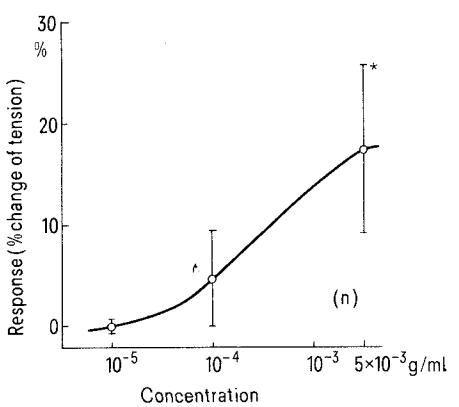

Figure 2. Dose-response curve relating pantethine concentration to increase in contractile force of guinea-pig papillary muscle preparations. Values are expressed as mean ± SEM. N = 6 in each group. * $p < 0.001$ vs 10^{-5} g/ml.

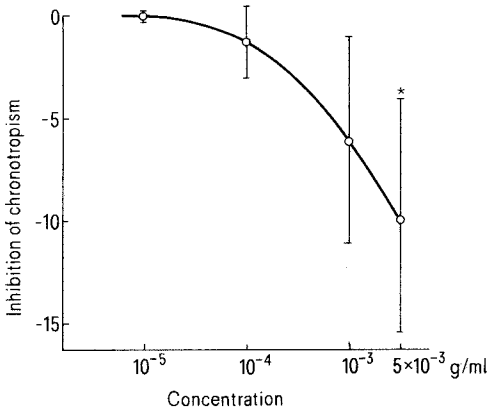

Figure 3. Negative chronotropic effect of pantethine in spontaneously beating guinea-pig atria. Values are expressed as mean ± SEM. N = 6 in each group. * $p < 0.02$ vs 10^{-5} g/ml.

in rats[9]. However, it is difficult to explain these positive inotropic and negative chronotropic actions of pantethine with respect to the quantity of endogenous co-enzyme A inside cardiac muscle. The mechanisms of these actions have not been elucidated as yet.

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Lead distribution in the nervous system of 8-month-old rats intoxicated since birth by lead

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Summary. Lead levels in the nervous system of rats intoxicated for 8 months by lead acetate (0.2% in drinking water) varied according to the region: the lowest levels were observed in sciatic nerve and the highest in hippocampus and cerebral neocortex, while intermediate levels were observed in pons medulla, cerebellum, midbrain, hypothalamus and striatum.

It has been shown that in immature rats intoxicated with high lead doses, the lead content was higher in the cerebellum than in other brain regions[2-4] and that this increase occurred before the appearance of morphological lesions[5]. The distribution of lead in the nervous system of immature or adult rats intoxicated with lower doses has been much less studied[6]. In intoxications with low doses, the morphological findings have been reported to occur only in discrete areas[7] and the various alterations in neurotransmitter levels or metabolism which have been observed varied according to the region of the nervous system[6]. We therefore measured the lead levels in several regions of the nervous system of chronically (35 weeks) intoxicated rats, as differences in lead content could possibly play a role in the development of functionnal abnormalities.

Methods. 48 rats of the Sprague Dawley strain were distributed at random on the first day after birth among the nursing dams (8 rats per dam). The dams were fed a commercial solid diet and, as drinking fluid, were given either a solution of 0.2% lead acetate (0.11% Pb) in freshly boiled deionized water (test animals) or freshly boiled deionized water only (control animals). During this period the test animals were thus intoxicated through the mother's milk. 21 days after birth, the pups were separated from the dams and grouped according to sex (7-9 animals in each group). They were given the same diet and drinking fluid as the mothers had previously received. The mean daily lead intake during the last month of intoxication was 30 mg for females, 44 mg for males.

Eight months after birth, 5 test and 5 control animals were implanted with electrodes on the dura, and electroencephalographic recordings under basal conditions were carried out 1 week later. Nerve conduction velocities (motor and sensory) were determined on the sciatic nerve of 20 other animals. 1 week later, the latter animals were anesthetized with ether, blood samples were collected by cardiac puncture and blood was washed from the vasculature by a perfusion of saline with heparin. The rats were then decapitated, the brains were excised and dissected out into: pons medulla, cerebellum, hypothalamus, midbrain, striatum, hippocampus and parietal cortex. The sciatic nerves were excised bilaterally, giving a length of about 1.5 cm. All the glassware (quartz or plastic) was nitric acid-washed and great care was taken to avoid contamination by atmospheric lead. Plasma, red cells and nervous system samples were digested in concentrated nitric acid at 100 °C in a multiblock heater (Labline Instruments). They were then diluted to 5 ml and analyzed by flameless atomic absorption spectrometry (Varian AA-1275 atomic absorption spectrophotometer fitted with a CRA-90 graphite furnace). The lead concentration was determined by adding aliquots of lead solutions of known concentration to each sample.

Results and discussion. The main features concerning test and control animals are given in table 1. Plasma, red cells, brain areas and sciatic nerve lead levels are shown in table 2. In control animals, lead levels in the blood and all nervous system regions were below the limit of detection given the technique used (0.05 µg/ml for fluid and 0.10 µg/g for solid samples. This high limit does not allow a comparison of our results with those published on lead distribution in normal brain[8,9]; however, our results are more in agreement with the studies reporting low lead contents[8-10] than with those reporting a very high content in some areas[11,12].

The lead content in the nervous system of test rats varied according to the region: the lowest in the sciatic nerve, similar intermediate levels in pons medulla, cerebellum, hypothalamus, midbrain, striatum; a higher content in the hippocampus and the highest in the parietal cortex. This distribution differs from that observed in immature animals treated with high lead doses, for which the highest lead content was constantly oberserved in cerebellum[2-5], or treated with low doses for which the highest content was in hippocampus[13]. It also differs from that of animals treated up to 8 weeks[13] in which the distribution was more equal. The high content observed in the cortex in our experiments (35-week treatment) is closer to those observed in dogs treated for 12 weeks where the highest level was in the occipital cortex[14], and to those observed in human lead encephalopathies where the highest level was observed in the hippocampal and frontal cortex[15] and in the cortical gray matter[16]. It is thus possible that in young animals the