approximate lethal dose for the oral route[6], there were no indications of an LD_{50} value (that dosage which will kill 50% of a test group of animals), nor of a dose response curve for oral doses. In order to determine what kinds of harmful effects to expect, and at what dosages they might be manifest, the decision was made to run experiments to assess both the LD_{50} value and the response curve. Due to the variations found in biologic entities, it is not generally considered meaningful to use the terms 'minimum lethal dose' or 'lethal dose' as once was customary. The aspect that is dealt with here is the LD_{50} value for single dose, oral administration.

Method. 16 Sprague-Dawley albino rats, 8 males and 8 females, were selected at random for these tests. The females ranged in weight from 150 to 250 g and the males varied from 350 to 425 g. In previous studies, a lowered resistance to Salmonellosis and chronic respiratory disease was noted. Consequently, stringent sanitary controls were put into effect and maintained during the experimental period. WEIL's[7] method of moving averages was the design utilized.

Procedure. The tetraethyl lead was obtained as a 98% pure solution from Ventor Corporation, Beverly, Massachusetts, USA. The lead compound was dissolved in 100% pure peanut oil to yield a concentration of 7.32 mg/ml. Each animal was weighed, the appropriate amount of solution was calculated and then pure oil was added to bring the volume administered up to 2 ml. Thus all animals received a similar dose of oil.

The animals were randomly assigned to 4 groups, except balancing was employed to assure an equal distribution of males and females in each group. Each of the groups was administered a different dosage level, with the logarithms of successive dosages differing by a constant (0.11394) according to the method set forth by WEIL[7]. Group I received a dose of 10.0 mg of compound per kg body weight, Group II received 13.0 mg/kg, Group III received 16.9 mg/kg and Group IV received 21.97 mg/kg. All doses were in a geometric progression with a factor of 1.3.

All animals were kept in the same light-cycled, temperature-controlled animals confinement room with their cages equally spaced on acceptable racks. 12-h light cycles were employed. Handling prior to the experimental period was restricted to one experimenter and no handling occurred between the administration time and time of death. The mortality data was collected over a predetermined, 14 day period of observation. The rats were visually examined daily for toxic signs, as well as for disease or parasites.

All dosages were administered by intragastric intubation of the 2 ml doses. Food and water were available in equal quantities to all groups. All rats were obtained from the supply house at a weight differential not to exceed $\pm$ 50 g. This was necessary in order to control for lead absorption differences due to amount of fatty tissue and to arrive at a narrow range of variance of dosages based on body weight. At no time during the 14 day period were the animals permitted to come into contact with sunlight or ultraviolet light, thus controlling for the highly variable absorptions of the compound from the blood serum which these factors may induce[8].

Results. The mortalities which resulted were 1 of 4 at 10.0 mg/kg, 0 of 4 at 13.0, 4 of 4 at 16.9 and 4 of 4 at 21.97. The mortality data was then matched to WEIL's tables. The following formula for computing the LD_{50} value was utilized: $LD_{50} = \log m \cong \log D_a + d(f+1)$ for $K = 3$. d was previously established as 0.11394 and the $\log D_a$ represents the log of the lowest of the four doses. Thus, with the f-value obtained from the tables, the LD_{50} value was found to be 14.18.

The estimation of the 95% confidence internal of this value is determined by the formula: $\sqrt{\log m} = d\sqrt{f}$. The LD_{50} and its confidence interval can thus be estimated as 14.18 (12.62 to 15.93) mg/kg. No curves are obtained using this method. It is planned to present these in a later paper.

Conclusions. All animals displayed neurological signs: lethargy, irritability and ataxia (muscular incoordination manifested when voluntary muscular activities are attempted) which represent an initial phase observed commencing with the second day. Violent jumping, trembling and thrashing following a loud noise plus aggressiveness and fighting appeared to represent a second stage in the progress of the toxicity. The aggressiveness was particularly manifest in Group III and IV males commencing with the fifth day. This was followed by convulsions, intermittent uncontrolled thrashing and, finally, death commencing with the sixth day. The three stages were observed in all groups (although death was not seen in one group) with the incipient stages appearing sooner and the progressive stages occurring faster in those animals receiving the higher doses.

Zusammenfassung. Im Hinblick auf den offensichtlichen Mangel eines LD_{50}-Wertes für Bleitetraethyl wurde bei intragastrischer Applikation im Rattenversuch der Wert 14,18 mg/kg (Spielraum 12,62–15,93) ermittelt.

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13 September 1971.

[5] The Merck Index: *An Encyclopedia of Chemicals and Drugs* (Ed. P. G. STECHER; Merck & Co., New Jersey 1968), 8th Edition, p. 1250.
[6] G. W. SCHEPERS, Arch. envir. Hlth. *8*, 277 (1964).
[7] C. S. WEIL, Biometrics *8*, 249 (1952).
[8] W. HUNG, Clin. Proc. Child. Hosp. *15*, 219 (1959).

Enhancement of Anaphylaxis of Isolated Smooth Muscle by Adenosine Phosphates and Inhibition of Anaphylactic Mechanisms by Adenosine-3′,5′-Cyclic Monophosphate

Anti-anaphylactic effect of adrenalin was shown to involve inhibition of antigen-mediated histamine release[1,2]. Also, it has been shown that histamine release is suppressed by theophylline[3]. Since both catecholamines and methylxanthines have distinct positive action on adenosine-3′,5′-cyclic monophosphate (cyclic AMP) system, it was assumed that their inhibitory actions on histamine release might be due to an increase of cyclic AMP: Recently, evi-

Effects of adenosine and its derivatives on anaphylaxis in vitro of smooth muscle from guinea-pig uterus

Compounds tested	No. animals	Histamine equivalent[a] of anaphylactic response in the presence of the compound tested	Hitamine equivalent[a] of control response	P	Influence (%)
Cyclic AMP[b]	7	10.2 ± 9.99[d]	9.47 ± 10.8[d]	>0.7	
Adenosine[b]	8	20.7 ± 14.9	9.62 ± 9.60	<0.05	+115
AMP[b]	9	4.74 ± 3.78	2.25 ± 1.83	<0.05	+111
Dibutyryl cyclic AMP[c]	9	1.35 ± 1.06	2.44 ± 2.14	<0.05	−45

[a] μg of histamine dihydrochloride added in 20 ml tissue bath. [b] 4.65 μ*M*. [c] 23.3 μ*M*. [d] Mean ± standard deviation.

dence supporting this hypothesis was obtained in the case of human leucocytes[3].

In the present experiments, the effect of cyclic AMP on anaphylaxis of smooth muscle was studied in vitro. Adenosine and adenosine-5′-monophosphate (AMP) also were studied, since the 6-amino group which is common to the purine nucleus of these compounds has been suggested to show a pharmacological activity on smooth muscles[4].

Materials and methods. Most of the procedures for the present study have been described in a previous paper[4]. Smooth muscle pieces for the in vitro anaphylaxis were obtained from the uterine horns of virgin guinea-pigs, and passive sensitization of the muscle pieces was made with anti-bovine serum albumin rabbit antiserum. For the study of the effects of AMP and dibutyryl cyclic AMP, a pool of antiserum which was different from the pooled serum for the initial series of experiments on adenosine and cyclic AMP was used. Concentration of the test compounds was 4.65 μ*M* in the tissue bath, only dibutyryl cyclic AMP being tested in a higher concentration of 23.3 μ*M*. The size of each reaction was expressed in the histamine equivalent of isotonic smooth muscle contraction obtained from the dose-response curve by histamine dihydrochloride on the individual muscle piece. From 1 animal, 4 smooth muscle pieces were used for an experiment: Averages of duplicate determinations with 2 pieces for control and 2 others for test, respectively, were calculated. Adenosine and AMP were obtained from Wako Pure Chemical Industries Ltd., Osaka (Japan); Cyclic AMPs were supplied by Sigma Chemical Co., St. Louis (USA).

Results and discussions. As shown in the Table, cyclic AMP, in a concentration of 4.65 μ*M*, failed to show an effect on the anaphylactic reaction. However, in the form of dibutyryl derivative which enters the cell more readily, and in a higher concentration, cyclic AMP inhibited the anaphylactic reaction. This inhibitory action may be attributed to the direct relaxing effect of cyclic AMP on smooth muscle and/or possible inhibition of histamine release by the compound, and is in favour of the hypothesis that the inhibitory actions on anaphylaxis of catecholamines and xanthines include the cyclic AMP system.

In contrast to cyclic AMP, adenosine and AMP in the concentration of 4.65 μ*M* enhanced the anaphylactic reaction. Thus, the marked intensification of anaphylactic reaction of smooth muscle by the same molar concentration of adenosine-5′-diphosphate (ADP) and adenosine-5′-triphosphate (ATP) observed in the previous study[4] seems to be related significantly to the pharmacological property inherent to the adenosine and its derivatives, cyclic AMP being the exception[5].

Zusammenfassung. Zyklisches 3′, 5′-Adenosinmonophosphat hindert in vitro die Anaphylaxis des glatten Muskels vom Meerschweinchenuterus, während Adenosin und Adenosin-5′-Monophosphat die anaphylaktische Reaktion erhöhen.

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[1] H. O. Schild, Q. Jl. exp. Physiol. *26*, 165 (1936).
[2] L. M. Lichtenstein and S. Margolis, Science *161*, 902 (1968).
[3] L. M. Lichtenstein, D. A. Levy and K. Ishizaka, Immunology *19*, 831 (1970).
[4] T. Okazaki, Experientia *28*, 49 (1972).
[5] Research supported in part by the Research-Aid Fund (1971) of the Prefecture of Fukushima. The authors are deeply grateful for the guidance of Prof. Takeo Yoshida.

Catalepsy Produced by Intraventricular Injection of Nicotine[1]

Catalepsy in rats[2], mice[2] and dogs[3,4] by subcutaneous or intravenous injections of nicotine has scarcely been studied. On the other hand, catalepsy by intraventricular injection of this drug in the conscious cat has not yet been studied. It was therefore of interest to know whether nicotine can produce catalepsy in conscious animals after intraventricular administration acting directly on the central nervous system. Furthermore, with the method of intraventricular application the brain structures close to or directly in contact with the ventricular surface can be implicated in the appearance of catalepsy.

Six cats of both sexes, weighing from 2.0 to 2.6 kg, were used in these experiments. For the injection of drugs into the cerebral ventricles a Collison cannulae was implanted aseptically into the left lateral ventricle during pentobar-

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[2] F. J. Nieuwenhuyzen, Proc. Koninkl. Akad. Wetenschap. *37*, 575 (1934).
[3] C. Gutierrez-Noriega, Rev. Neuro-Psiquiat. *5*, 323 (1942).
[4] M. Beluffi, Riv. sper. Freniat. *76*, 83 (1952).