tion by EDTA is not specific for lead. There is a definite order of chelation which varies for a given di- or tri-valent ion with the pH of the solution(3). Popovici *et al.*(6), have shown that the rapid intravenous injection of the non-chelated EDTA in rabbits produced tetany and death from hypocalcemia. By administering the compound as the calcium chelate, this danger is eliminated and toxicity is minimal.

The cases presented demonstrate that CaEDTA was well tolerated and effective in producing significant increases in the excretion of lead by the kidney. Those patients manifesting symptoms due to lead ingestion noted a prompt alleviation of symptoms. The increase in urinary lead excretion consequent to oral administration of CaEDTA was quantitatively less and maximal excretion was attained more slowly than when CaEDTA was given intravenously. Gastro-intestinal absorption of CaEDTA was suggested by the increase in urinary lead excretion following oral administration; however, the formation of the lead EDTA complex in the gastro-intestinal tract with subsequent absorption cannot be ruled out. Mosey and coworkers have shown that the lead EDTA complex when taken by mouth was rapidly absorbed from the upper gastro-intestinal tract(7). Further study is needed to clarify this point.

Summary. Seven patients with abnormally elevated blood lead and/or urinary lead excretion values are presented who received CaEDTA orally, intravenously, or by both routes. This organic chelating agent effected a marked increase in urinary lead excretion by both routes. Intravenous administration was more effective than oral therapy. Preliminary results indicate that CaEDTA is the most effective agent proposed for the treatment of plumbism. Side-reactions were minimal with the dosage schedule employed.

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Comparison of Effects of Various Phosphate Compounds and Aluminum Silicate on Isolated Frog Heart. (20074)

JEAN M. MARSHALL AND E. COWLES ANDRUS.*
(With the technical assistance of Barbara Rayboen.)
From the Departments of Physiology and Medicine, Johns Hopkins Medical School, Baltimore, Md.

Various investigators, Linder and Rigler(1), Sheikhon(2), Lichtneckert(3), Loewi(4), have noted the restorative effects of adenosine triphosphate on the hypodynamic frog heart. A great variety of surface active substances such as sodium oleate, animal charcoal, camphor and serum are also known to increase the amplitude of contraction of the hypodynamic frog heart, Wieland(5), Clark(6). The present experiments were undertaken to study the effects of various phosphate compounds, ATP, ADP and sodium tripolyphosphate(TPP) on the hypodynamic frog heart and to compare them with a surface active substance, aluminum silicate (bentonite).

Methods. The heart of a frog, *Rana pipiens,* was removed, suspended in oxygenated Ringer solution† in a glass muscle cham-

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† NaCl 0.65 g %, KCl 0.014 g%, $CaCl_2$ 0.012 g %, adjusted to pH 7.3 with phosphate buffer.

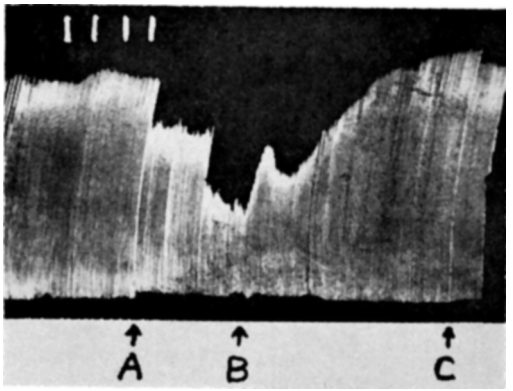

FIG. 1. Effect of ATP on rhythmically driven frog ventricle in ½ Ca-Ringer. A—addition of ½ Ca-Ringer. B—addition of .20 mM ATP. C—30 min. after addition of ATP. Time in 15 sec. intervals. Interval between A and B, 3 min. Interval between B and C, 30 min.

ber at 25°-27°, and the mechanical response of the ventricle recorded according to the method described by Clark(7). The ventricle was stimulated by shocks from a square-wave stimulator, and the contractions were recorded on a kymograph. The general procedure in all experiments consisted of 1) an initial control period of about 15 minutes with the ventricle in normal oxygenated Ringer solution, 2) a depression period in which the amplitude of contraction was lowered to 50% of the control level by one of the procedures described below, 3) a period in which the test substance was added, 4) a final period in which the ventricle was returned to normal Ringer. All values for recovery of amplitude of contraction are expressed as % of the initial control level.

Results. 24-27 experiments with each of the 4 compounds at various concentrations were done in which the ventricles stimulated at 22-60 per min were depressed by alteration of the perfusion fluid in one of the following respects: lowered calcium, increased potassium, N_2 plus IAA, or acetyl-β-methylcholine chloride. A typical kymograph record of the characteristic recovery pattern appears in Fig. 1. In general the amount and duration of recovery varied directly with the concentration of test substances added, up to a maximally effective concentration. Fig. 2 graphically summarizes the data from 24 experiments with various concentrations of ATP in the presence of ½ Ca Ringer. From curves A and B it can be seen that the degree and duration of recovery increases with increasing concentrations of ATP up to a maximum concentration of 0.19 mM. No correlation was found between the rate of stimulation and the degree of recovery. Table I summarizes the data from the 4 groups of experiments. The figures listed in this table represent average values for the degree and duration of recovery at maximal effective concentrations of the test substances.

Five experiments were done with calcium-free Ringer as the perfusion fluid. In all of these experiments the amplitude diminished to about 10% of the control level, and the addition of ATP, 0.30 mM, restored the amplitude of contraction to 80% of the control level.

The excitability of the ventricles was tested by recording their ability to respond to double the initial frequency of stimulation in the presence of Ringer solution containing six times the normal potassium content (0.084 g %). During the initial control period with normal Ringer in the cannula, the frequency of ventricular contractions followed exactly when the rate of stimulation was doubled. After replacement of the normal Ringer with the 6-fold K, the amplitude of contractions diminished and there were frequent dropped beats. With the addition of ATP (0.19 mM) or ADP (0.22 mM), the amplitude of con-

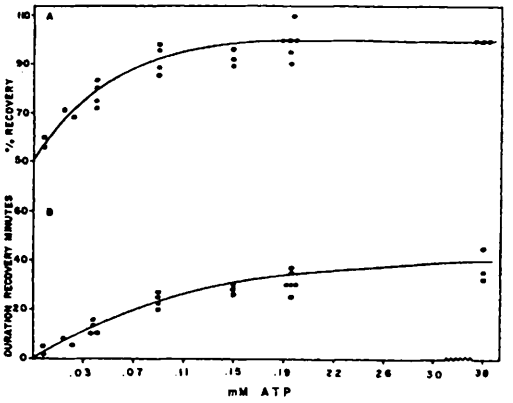

FIG. 2. Restorative effects of ATP on isolated frog ventricle in ½ Ca-Ringer. Curve A—effect of conc. of ATP on degree of recovery of amplitude of contraction. Curve B—effect of conc. of ATP on duration of recovery.

TABLE I. Comparison of Relative Effectiveness of ATP, ADP, TPP, and Bentonite at Maximal Effective Concentrations.

Solution	Test substance	Conc., mM	Recovery, %	Duration, min.
½ Ca Ringer .006 g %	ATP*	.19	100	35
	ADP*	.22	90	20
	TPP†	.27	70	90
	Bent.‡	.4 ml	85	9
4 K Ringer .056 g %	ATP	.19	100	30
	ADP	.22	79	19
	TPP	.32	80	60
	Bent.	.4 ml	60	5
N_2 + IAA 1:10000	ATP	.28	75	3
	ADP	.33	0	0
	TPP	.54	0	0
	Bent.	.6 ml	0	0
Acetyl-β-methylcholine chloride "mecholyl" 1:1000	ATP	.02	87	30
	ADP	.03	74	30
	TPP	.27	57	15
	Bent.	.4 ml	70	15

* Sodium salts of ATP and ADP were prepared by Sigma Chemical Corp., St. Louis, Mo.

† $Na_5P_3O_{10}$ kindly supplied by Victor Chemical Works, Chicago, Ill.

‡ Powdered aluminum silicate: SiO_2 43.66%, Al_2O_3 41.26%, Fe_2O_3 trace, CaO .86%, MgO .12%, loss on ignition 13.99%. Kindly supplied by Whittaker, Clark and Daniels, New York City.

traction returned to the control level and the rate of contraction followed the rate of stimulation precisely when the latter was doubled (10 exp.). TPP and bentonite were ineffective under these conditions.

All 4 compounds were effective in bringing about recovery after depression by ½ Ca, 4 K, and acetyl-β-methylcholine chloride. In general ATP was the most effective of these compounds. This observation is substantiated by the following facts:

ATP further increased the degree of recovery beyond that attained with TPP and bentonite in the ½ Ca and acetyl-β-methylcholine chloride experiments listed in Table II.

In the experiments with N_2 plus IAA, only ATP was effective in restoring contraction. Although this restoration was extremely transitory (3 minutes) it could be continued by repeated additions of ATP to the cannula. Under these conditions ATP is presumably acting metabolically by virtue of its terminal pyrophosphate. Korey(8) observed that ATP and ADP would initiate contraction of an isolated muscle fiber preparation whereas adenosinemonophosphate and inorganic pyrophosphate were ineffective. Recently, Greiner (9) reported a fall in ATP concentration concomitant with the decline of contractile force in anoxic cat papillary muscle.

ATP, and also ADP, proved effective in restoring excitability in hearts whose excitability had been depressed by excess potassium. Acierno, *et al.*(10) found that ATP restored excitability toward the normal level in hearts whose excitability was depressed by acetylcholine.

The data presented here appear to indicate that ATP can act in two ways under the conditions of these experiments, 1) by virtue of its being a linear polyphosphate and possessing a certain amount of surface activity, 2) by virtue of having a terminal "high energy" phosphate bond. Evidence for the first instance is found in the results of the ½ Ca, 4 K and acetyl-β-methylcholine chloride experiments in which ATP, ADP, TPP and bentonite all exerted restorative effects. On the other hand, in the experiments in which the energy metabolism was inhibited by nitrogen and IAA, ATP alone was effective in

TABLE II. Effects of ATP in the Presence of TPP and Bentonite.

Solution	Substance	Conc., mM	Recovery, %	Substance	Conc., mM	Recovery, %
½ Ca Ringer	TPP	.160	59	ATP	.12	88
	"	.03	52	"	.02	85
	"	.09	56	"	.06	89
Acetyl-β-methylcholine chloride "mecholyl"	"	.32	54	"	.02	78
	"	.27	54	"	.02	72
	"	.13	55	"	.02	85
	"	.03	50	"	.02	87
	Bent.	.4 ml	72	"	.24	80
	"	.2 "	59	"	.24	82
	"	.1 "	52	"	.24	82
	"	.1 "	52	"	.06	85

restoring contractions. Here, ATP was presumably acting by virtue of its possession of "energy rich" phosphate bonds.

Summary. ATP, ADP, sodium tripolyphosphate and aluminum silicate restored the amplitude of contraction in hearts made hypodynamic by reduced calcium, excess potassium, or acetylmethylcholine chloride. In general, ATP was the most effective compound. ATP alone was effective in hearts depressed by N_2 plus IAA. ATP and ADP restored the excitability in hearts depressed by excess potassium. None of the 4 compounds had any effect on the freshly excised heart perfused with oxygenated Ringer.

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Effect of Synthetic Lysine Polypeptides on Rabbit Blood Coagulation.* (20075)

J. R. RUBINI,† R. R. BECKER, AND M. A. STAHMANN.

From the Department of Biochemistry, University of Wisconsin, Madison, Wisc.

As part of studies of the biological properties of lysine polypeptides(1-5), an investigation of their effect upon blood coagulation was undertaken in 1950. Since the basic polypeptides of lysine had been shown to resemble the protamines in many of their chemical and biological properties, it was expected that they would also have an effect on blood coagulation similar to that of the protamines. During the course of our investigations, de Vries, Schwager, and Katchalski reported on their observations of the action of some water-soluble poly-α-amino acids on blood clotting (6). In view of the continued interest in synthetic polypeptides and blood coagulation (7,8), it seems desirable to report some of our results.

Experiments with synthetic polypeptides of L-lysine and L-glutamic acid were carried out to study their effects on the coagulation of rabbit blood *in vitro* and *in vivo* and compared with protamine and heparin. While human and sheep blood cells have been found to be agglutinated by very low concentrations of lysine polypeptides(3) and also protamine (9), these substances do not agglutinate rabbit red cells. Rabbit blood was therefore used because it provides a good experimental system in which to study coagulation without any complicating effects of agglutination. The results of some of these experiments indicate that lysine polypeptides prolong the whole blood clotting time, increase the prothrombin time and neutralize the effect of heparin.

Materials and methods. The polypeptides were synthesized by polymerization of the N-carboxyamino acid anhydrides and analyzed by reaction with nitrous acid to determine the average number of amino acid residues (units) per polypeptide molecule(10,11). Stock solutions were made up in 0.85% saline,

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† Present Address: Western Reserve University School of Medicine, Cleveland, O.