Specific intranuclear fluorescence with the anti-J.W. macroglobulin was demonstrable in cells of one other patient with macroglobulinemia. Recently Curtain(10), using a similar technic, observed cytoplasmic, but not intranuclear, fluorescence of plasmacytoid cells from bone marrow of 2 patients. One of these patients had increased serum gamma macroglobulins but an atypical clinical history for Waldenström's macroglobulinemia(11). The other patient appeared to have multiple myeloma.

Summary. Immunofluorescent and cytochemical technics were used to identify 18S gamma macroglobulin within the nucleus and cytoplasm of lymphocytoid plasma cells from a patient with macroglobulinemia of Waldenström. Intranuclear fluorescence coincided with presence of intranuclear PAS positive protein. This observation supports the concept, suggested by histochemical studies, that circulating macroglobulin and intranuclear protein of lymphocytoid plasma cells are closely related and may be identical. The evidence suggests that macroglobulin is formed in lymphocytoid plasma cells.

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Response of Isolated Auricles to Some Substances of Metabolic Interest.* (25556)

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Inotropic actions of various intermediary metabolites have been studied most often using preparations of rat ventricular muscle (1,2,3), papillary muscle of cat(4) or isolated heart(5,6,7). Similar studies on isolated auricles of rabbit(8) have been conducted in a somewhat different manner and differences in activity noted. Ventricles and auricles also differ with respect to their sensitivity to various inorganic ions(9,10) and exhibit a difference in responsiveness to stimulation of their extrinsic nerve supply(11). These observations led us to perform the following experiments to determine whether or not the auricular effects in the rat of malonic acid and certain intermediates of the tricarboxylic acid cycle are similar to those elicited by these agents in ventricular preparations from the same animal. We also attempted to explain the observed effects utilizing a somewhat different experimental approach than that employed previously.

Materials and methods. Albino rats of Holtzman strain, unselected as to sex and weighing approximately 150 g were used. Animals were anesthetized with ether and the hearts rapidly removed. After excising ventricular muscle, blood vessels, fat and connective tissue, the auricles were suspended in a

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muscle chamber containing 250 ml of Locke's solution maintained at constant temperature and aerated with mixture of 95% oxygen and 5% CO_2 by means of sintered glass plate at bottom of chamber. The Locke's solution had the following composition: NaCl, 0.9%; KCl, 0.042%; $CaCl_2$, 0.024%; $NaHCO_3$, 0.05%. In those experiments employing a glucose-containing medium, the concentration of this substance was 0.10% unless otherwise stated. Contractile amplitude and rate of spontaneous beat were recorded with isotonic lever on a kymograph. Auricles were allowed to equilibrate at least one hour after placing in the medium prior to addition of any of the compounds and were washed frequently after each response. Compounds were employed as obtained from commercial sources, dissolved in medium used in muscle bath, adjusted to pH 7.0, and added to bath in a volume not exceeding 2 ml.

Results. The isotonic contractile amplitude of auricles of the rat was reduced by various intermediary metabolites and malonate (Table I). The effect produced by acetate was more transient than that seen with other compounds. Although addition of glucose (5 or 10 μmol/ml) to the medium increased contractile amplitude at 28°C, it caused little or no change in contractility of auricles studied at 38°C. Inhibitory activity was greatest with citrate and least with lactate. In general a negative inotropic response was accompanied by decrease in rate, though of less magnitude, and the effects produced by the compounds in a medium maintained at 28°C were very much like those at 38°C.

TABLE I. Negative Inotropic Effects of Various Compounds on Isolated Rat Auricles.

Compound*	Conc. of compound (μmol/ml)			
	1	5	10	20
	% decrease in contractile amplitude			
Citrate	69 ± 2.1†	100 ± 0	100 ± 0	100 ± 0
Malonate	7 ± 1.0	22 ± 4.0	44 ± 3.4	90 ± 6.9
Malate	15 ± 2.0	25 ± 3.9	54 ± 7.0	18 ± 2.0
Succinate	10 ± 3.0	28 ± 2.9	58 ± 5.2	73 ± 6.4
Pyruvate	4 ± 2.6	20 ± 4.3	33 ± 7.9	40 ± 5.0
Fumarate	0	15 ± 4.7	25 ± 1.3	41 ± 2.2
Acetate	0	8 ± 1.6	36 ± 5.3	45 ± 3.6
Lactate	0	6 ± 1.6	24 ± 4.2	39 ± 4.2

* All compounds were employed as sodium salts.
† Mean ± S.E.

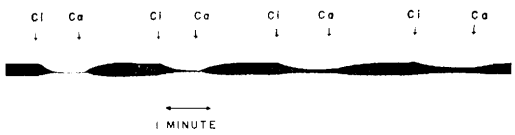

FIG. 1. Effect of repeated alternate administration of calcium and citrate on contractile amplitude of isolated auricles of the rat. Following the response to 2.5 mmol of sodium citrate (Ci), 2.25 mmol of calcium chloride (Ca) was added. Seven such titrations were performed before signs of calcium intoxication appeared.

Degree of inhibition of contractile amplitude was not significantly affected by addition of glucose to the medium. Sodium malonate produced a reduction in amplitude of contractions of the auricles. This was seen in a medium containing either 2.45 μmol/ml or 2.16 μmol/ml of calcium chloride. The effects were very similar at 28°C and 38°C and were not significantly affected by addition of glucose to the medium. Addition of sodium (as sodium chloride) equal in amount to that in the compounds under study did not alter contractile amplitude.

Negative inotropic effect of the metabolites was reversed by addition of appropriate amounts of calcium chloride to the medium. Reciprocally, enhanced contractility produced by certain quantities of calcium was antagonized by addition of metabolites under study. Using the "titration" method of Swain *et al.*, (13), experiments were performed in which calcium chloride and one of the metabolites were alternately added to the bath. One such experiment is illustrated in Fig. 1 where 2.5 mmol of sodium citrate was "titrated" in this manner with 2.25 mmol of calcium chloride. A series of 7 such "titrations" could be performed before signs of calcium toxicity were observed. Similar "titration" experiments were conducted using succinate, malonate, malate, fumarate, pyruvate and lactate. In each experiment the following were determined: (1) lowest concentration of calcium necessary in the medium to cause a just perceptible reversal of the negative inotropic effect produced by 2.5 mmol of the compound used, (2) concentration of calcium required for complete reversal of the effect produced by the compound, and (3) total calcium concentration present in the medium during a

TABLE II. Reversal of Negative Inotropic Effects of Various Compounds on Rat Auricles by Calcium Chloride.

Compound*	$CaCl_2$ conc. (μmol/ml) producing Partial reversal	Complete reversal	Toxic effects
Citrate	3.0	9.0	65.2
Succinate	.4	.8	7.0
Malonate	.1	1.0	6.2
Malate	.2	.6	6.4
Fumarate	.2	.7	7.1
Pyruvate	.2	2.0	8.4
Lactate	.2	.8	6.2

* All compounds were employed at a final concentration of 10 μmol/ml except lactate in which case concentration was 20 μmol/ml.

"titration" experiment when toxic effects of calcium, *e.g.* arrhythmias, first appeared. These results are summarized in Table II. Calcium was least effective in counteracting the negative inotropic response to citrate. It also will be noted that citrate was capable of antagonizing approximately 10 times more calcium than were the other compounds.

Discussion. These experiments demonstrated that all compounds examined, except glucose, exert a negative inotropic effect on the isolated auricles of the rat. These results confirm those obtained by a previous investigator (8) who employed the isolated auricles of the rabbit. Similar effects have been observed on isolated perfused rat heart with a glucose-free perfusing medium(6) and on the isolated perfused rabbit heart(7). The increase in amplitude observed after addition of glucose at 28°C parallels the observations of previous investigators(4) on papillary muscle of the cat. However, in contrast to these findings, it has been reported(1) that hypodynamic rat ventricular strips show recovery of contractile amplitude upon addition of pyruvate, lactate, β-hydroxybutyrate, acetate, succinate or glucose in phosphate buffered solutions. This difference in results may be related to a difference in sensitivity of auricles and ventricles to these compounds or to differences in media or technics employed since the effect of succinate, at least, varied with different media employed in these experiments. Hydrogen ion concentration also appears important since at pH 7.6 in a bicarbonate buffered medium, no recovery of depressed amplitude occurred with citrate, succinate or malate on rat ventricular strips(3) whereas the latter 2 substrates were effective in a medium buffered at pH 6.2. Positive inotropic actions of pyruvate, glucose and acetate but not citrate, fumarate, succinate and malate have been observed with hypodynamic rabbit auricles(8).

The negative inotropic effect of malonate which we observed is in contrast to lack of activity of this compound on the driven auricular preparation reported by other investigators(12) and positive inotropic activity on rat ventricular strips seen by still other workers(2). The latter investigators employed a higher concentration of calcium (2.45 μmol/ml) than we used. However, even at this higher calcium concentration, we observed only negative inotropic responses of rat auricles to malonate. The results suggest that reduction of contractility by malonate is not the result of inhibition of metabolic activity in the tricarboxylic acid cycle, since metabolites of the cycle are capable of producing similar negative inotropic responses, as reported previously(8).

With the exception of acetate, all compounds utilized in this study which elicited negative inotropic responses are theoretically capable of forming chelate-type compounds with the calcium ion. Sodium acetate, a monodenate compound, would not be expected to chelate with calcium but complexes of a nonring-type might be formed. Our data suggest that the negative inotropic activity of the compounds examined could be due to a decrease in concentration of available calcium in the medium. This concept agrees with the work of other investigators who believe that depression of amplitude by malonate(2) and by citrate(8) is probably due to a reduction in calcium concentration of the medium. The myocardial depressant effects of compounds employed were overcome by addition of appropriate amounts of calcium chloride to the medium. Conversely the toxic effect of certain concentrations of calcium chloride could be limited by subsequent addition of one of the compounds in question. "Titration" experiments provide even more convincing support for the view that there is some interaction between calcium ion and these com-

pounds. Reversal of only a single episode of depression with calcium would not be significant since the negative inotropic action of many substances can be antagonized nonspecifically with calcium salts but several consecutive reversals provide more definitive information(13). It was reasoned that if a complex were formed between the compound in question and calcium, most of the calcium would be bound in an inactive form and alternating additions of calcium and the compound would result in alternating increases and decreases in contractile amplitude. On the other hand if no such complex were formed and the response to calcium were specific then evidence of calcium toxicity should appear early as a result of an increased concentration of calcium ion in the medium. In the absence of compounds studied, a concentration of approximately 3 μmol ml of calcium chloride in the medium proved toxic as evidenced by appearance of arrhythmias. Concentrations of calcium 2 or more times this value were required to produce arrhythmias when one of the compounds was in the medium (Table II). We are unaware of any report where an auricular depressant and calcium have been "titrated" in the manner described where there is more than enough calcium ion to cause toxic signs yet none are seen. Therefore, it appears that a part of the calcium has been converted to an inactive form and that the negative inotropic action of the compounds studied is related to their capacity to bind or inactivate calcium.

Although it would be of interest to relate dissociation constants of calcium complexes of these compounds to the capacity of calcium to reverse their toxic effects, there are such wide variations reported for constants by various investigators that it appears unwise, if not impossible, to attempt to do this. Ranges of reported values overlap to a considerable extent and the small differences observed correlate to a certain extent with small differences in calcium concentration required for toxic effects during "titration" experiments.

Summary. Various compounds of metabolic interest, both substrates (citrate, succinate, fumarate, l-malate, pyruvate, dl-lactate, acetate) and an inhibitor (malonate), produced a negative inotropic effect on isolated auricles of the rat. Of the intermediary metabolites the greatest degree of negative inotropic activity was exhibited by citrate and the least by acetate and lactate. Negative inotropic activity of these compounds did not appear to be significantly affected by temperature or addition of glucose to the medium. Results obtained following addition of calcium chloride to the medium, after effects of test compounds were established as well as results from "titration" experiments, suggest that the effects of metabolites and antimetabolite studied may be related, at least in part, to their capacity to form complexes with calcium.

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