was 0.01 to 0.02 μg/ml and with time the threshold decreased 10-fold(6); in our work the threshold for non-atropinized rat colon was 0.02 μg/ml of salt (approximately 0.01 μg/ml as base).

It should be noted that our measurements were obtained at room temperature, a point of considerable convenience, although presumably the sensitivity would be greater at 37°. Rat colon is thus a useful tissue for such studies.

Summary. Strain gauge tensometers applicable to the study of ionic or metabolic exchanges in smooth muscle strips have been developed. Sensitivity was assessed using rat colon with carbachol, Substance P, and serotonin. The threshold for carbachol was 4 mμg/ml, for Substance P 0.01 to 0.03 unit/ml (vonEuler units) and for serotonin as salt 0.02 μg/ml.

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Effect of Malonate on Performance and Metabolism of Isolated Dog Heart.* (23875)

GEORGE FAWAZ, BERIN TUTUNJI AND EVA N. FAWAZ
Department of Pharmacology, School of Medicine, American University of Beirut, Lebanon

Malonate is the classical inhibitor of the Krebs tricarboxylic acid cycle. Krebs and Eggleston(1), using pigeon breast muscle mince, found that 0.025 M malonate caused 92% inhibition of pyruvate oxidation. Smyth (2) used the same concentration of malonate in sheep heart mince and observed an 80% inhibition of pyruvate oxidation. If malonate were added in sufficient concentration to dog heart-lung preparation one would expect to produce heart insufficiency. For if it is assumed that oxidative metabolism in the heart is mainly channeled through the Krebs cycle, an interruption of the latter would lead not only to inhibition of oxidation, but also, according to current concepts, to cessation of production of high-energy phosphate bonds which, directly or indirectly, provide energy for muscle contraction. Early experiments with 0.03 M malonate produced a mild insufficiency lasting a few minutes, followed by a return to normal performance. In a few instances, there was even a "rebound" and a greater heart output was observed than before malonate addition. This unexpected result could have several explanations, 1) that malonate did not diffuse into heart muscle in sufficient concentration to inhibit succinate oxidation. Succinate determinations were then performed on malonate-treated hearts, an accumulation of succinate being interpreted as evidence for diffusibility of malonate.

Methods. Dog heart-lung preparations were performed under pentobarbital anesthesia but blood donors were anesthetized with chloroform. 5.6 g Na_2 malonate H_2O were dissolved in 50 cc H_2O and added to venous reservoir in 10 minutes. This brings molarity of the system to 0.03, if total weight of preparation is taken as 1 kg. Where double dose was used 5 minutes elapsed and another dose of 5.6 g malonate in 50 cc water was introduced in 20 minutes. Experiments were terminated 65 min. after onset of first malonate infusion. Phosphorus compounds were estimated as previously described(3) except

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that 7 instead of 10-minute hydrolysis in 1N HCl at 100°C was used to split off two-thirds of ATP-phosphorus. Phosphorus values are expressed as percentages of total acid-soluble phosphorus. By coincidence, the average total acid-soluble phosphorus is just a little over 100 mg phosphorus/100 g heart muscle tissue. Succinic acid was determined by enzymatic method of Szent-Györgi and Gozsy(4). However, some important precautions must be taken. a) Permanganate to destroy malonate should be applied not to original protein-free filtrate, as in earlier work of Krebs(1), but to the residue of ether extract derived therefrom, otherwise too high values for succinate are obtained. In dog serum, the difference can be of the order of several hundred percent. b) Perfect recovery of succinate is obtained if added to protein-free filtrate of muscle, but if added to a chilled aqueous suspension of muscle powder before deproteinization, recovery is incomplete. Recovery can be improved to 85-90% if the aqueous suspension containing added succinate is well mixed with excess sulfuric acid before tungstate is added. This procedure was mainly followed. Addition of succinate to sulfuric acid solution before introduction of tissue and tungstate leads to satisfactory recovery. Excised piece of heart tissue is frozen quickly in CO_2-snow, weighed, then pulverized in CO_2-snow. The powder is transferred quantitatively to glass-stoppered flask containing 7.75 ml glass-redistilled water and 0.8 ml 1:1 H_2SO_4/g of tissue. Shake well for 2 minutes then add 0.5 ml 10% sodium tungstate and continue shaking in the cold 10 minutes. Centrifuge and use 15-20 ml for extraction with ether in Kutscher-Steudel apparatus. The ether extract, to which 0.6 ml 0.1 M phosphate buffer of pH 7.4 is added, is evaporated and last traces of ether are removed by evacuating in desiccator for 15 minutes. Transfer to Kutscher-Steudel extractor using a total of 12 ml warm water, add 1 ml 1:1 H_2SO_4 and 2 ml 5% permanganate and mix. Place in water bath previously heated to 80°C for 30 min. at end of which water cools off to 50°C. Add another 1 ml 1:1 H_2SO_4, mix and add ether gradually while mixing. Again, 0.6 ml phosphate buffer is added to ether extract and the ether distilled off. Neutralize aqueous residue with NaOH using phenol red. Dry completely in vacuum desiccator over H_2SO_4 and take up the residue in 0.6 ml warm water. Use 0.3 ml of solution for enzymatic determination with succinoxidase.

Results. The "resting" succinate values in heart tissue, as determined in open-chest animals with artificial respiration show considerable variation. In 12 animals, the values ranged from 0.0 to 0.12, an average 0.05 $\pm$ 0.011 mg succinic acid/g wet tissue. In 8 control heart-lung preparations for 65 min. the average succinic acid content was 0.06 $\pm$ 0.013 mg/g (Table I). In 0.03 M malonate preparations the succinic acid content was 0.243 $\pm$ 0.033 mg/g. The difference is significant. The succinate accumulation with double dose of malonate is 0.29 $\pm$ 0.016. Succinate values reported by Krebs and Eggleston(1) and Smyth(2) are probably too high since these authors did not use double extraction procedure. These results do not prove, but constitute presumptive evidence that malonate diffused into heart muscles. Experiments by Busch and Potter(9) on intact rats provide direct evidence that malonate freely diffuses into rat heart *in situ*.

Table I shows that up to one hour, 0.03 M malonate had no significant effect on performance of the isolated heart as judged by changes in minute output and venous pressure. Only during addition of malonate, there was a transient mild insufficiency, probably not specific for malonate since it can also be induced by succinate. In 5 malonate experiments, Krayer's "competence index" (5) was also measured at beginning and end of experiment and in 4 of these, the index remained unchanged. Only in one case was there a change from 0.9 to 0.7. There is no correlation between succinate accumulation and heart performance, or between succinate accumulation and level of high-energy phosphorus compounds, which also remained within normal limits.

The results obtained with double dose of malonate (0.06 M) are partly reproduced in Table I. Before infusion of second dose was complete, there was in each case a sudden dilatation of the heart with significant de-

TABLE I. Effect of Malonate on Performance and Metabolism of Dog Heart-Lung Preparation. Duration of experiments, 65 min. (Phosphorus values expressed as % of total acid-soluble phosphorus.)

% change in systemic output at end of exp.	Change in venous pressure at end of exp. (cm of H_2O)	Succinic acid, mg/g heart tissue	Phospho-creatine-P	Inorganic-P	Labile nucleotide-P
		Controls			
−20	+ .7	.04	24.7	13.0	31.0
0	+ .1	.08	23.1	16.2	31.2
0	.0	.09	18.4	16.0	29.6
+ 1	− .4	.12	24.0	13.0	31.3
− 4	.0	.07	18.6	16.7	30.2
0	+ .6	.02	17.7	20.6	28.0
+25	+ .5	.02	20.5	14.6	35.1
+ 1	+1.1	.03	24.3	15.2	28.9
	Mean ± S.E.	.06 ± .013	21.4 ± 1.03	16.4 ± .91	30.7 ± .76
		.03 M malonate			
+ 5	+ .1	.20	29.4	14.7	29.3
− 1	+ .1	.43	21.0	11.6	34.8
+10	+ .2	.44	21.5	13.0	32.4
+ 6	− .1	.18	29.8	10.7	29.2
−10	+ .1	.12	26.2	14.5	31.6
− 6	+ .2	.13	21.9	18.4	29.4
− 7	.0	.18	24.2	17.9	28.2
0	+ .2	.19	26.7	15.1	26.9
− 6	+2.6	.44	17.4	16.9	30.0
− 4	.3	.45	20.6	17.8	27.7
−27	+1.5	.59	10.3	21.7	30.3
− 2	− .6	.02			
− 6	+1.1	.14			
− 3	+ .1	.22			
− 3	+3.1	.28			
− 9	.0	.08			
− 5	+ .1	.06			
− 9	+ .3	.31	29.2	12.5	28.6
+22	+ .2	.15	28.6	12.9	28.5
	Mean ± S.E.	.243 ± .033	23.6 ± 1.6	15.2 ± .88	29.7 ± .6
		.06 M malonate			
−56	+1.2	.46	21.8	17.6	29.6
−78	−1.1	.14	28.3	12.4	26.8
−34	.0	.32	24.0	14.6	29.9
−69	−1.7	.36	29.0	9.9	30.2
−13	+1.5	.26	24.0	13.1	29.4
−56	+2.6	.22			
	Mean ± S.E.	.293 ± .016	25.5 ± 1.4	15.5 ± 1.6	29.2 ± .19

crease in rate. ECG changes were also observed such as prolongation of QT, electrical alternans and frequent multifocal premature ventricular systoles. Level of blood in the venous reservoir fell substantially owing to heart dilatation. The reservoir was not raised to restore venous inflow level for fear of intensifying the arrhythmias, so that the observed lack of significant rise in venous pressure, and the decrease in systemic output are of little value in assessing cardiac competence. Significantly, phosphorus values are unchanged, the phosphocreatine values, as in 0.03 M malonate experiments, are even slightly higher than in control experiments (3).

Discussion. One purpose of the present investigation is to compare the actions of malonate *in vitro* and *in vivo*. It is shown that concentration of malonate (0.03 M) which according to Krebs and Eggleston(1) and of Smyth(2) almost completely interrupts the citric acid cycle in muscle mince, has no effect on performance of heart-lung preparation or on phosphorus compounds of muscle, despite accumulation of succinate. Results obtained

by treating the heart-lung preparation with double dose of malonate are difficult to interpret. Succinate values are only slightly higher than with the single dose, and the high energy phosphate values are unchanged. There is thus no correlation between succinate accumulation and heart competence. The "failure" observed with the double dose was sudden, appearing before the whole dose was added, and was characterized by significant ECG changes. It may not be due to interruption of the Krebs cycle. It could well be due to hypertonicity of the serum, or binding of certain cations by malonate, or some unknown effect on myocardium. It has already been shown, for instance, that heart failure due to dinitrophenol cannot be attributed to its known action of uncoupling phosphorylation and oxidation(10). Other authors using different preparations have also noted an "anomalous" behavior of heart muscle to malonate(6,7,8).

Our experiments do not prove that succinate oxidation is not essential for cardiac function or that alternative pathways are functioning in malonate treated hearts. Malonate inhibition in our system may not be complete and succinate oxidation may not be the rate limiting step in the Krebs cycle. Further experimentation is necessary.

We have no explanation for variations in succinate accumulation after malonate. Preliminary experiments indicate that nutritional state of animals is of no significance.

Summary. Malonate in a concentration (0.03 M) which almost completely blocks the Krebs cycle in muscle mince, has no significant effect on performance of the isolated dog heart (heart-lung preparation) or on its high-energy phosphorus compounds. 0.06 M malonate produces also no change in the phosphorus compounds, there is even a slight increase in phosphocreatine. The double dose of malonate, however, produces a sudden cardiac dilatation before the full amount is administered. This is accompanied by a decrease in heart rate, ECG changes and arrhythmias. Significant increases in heart succinate with both doses constitute presumptive evidence that malonate diffuses into heart muscle but this accumulation of succinate shows variations and it cannot be correlated with cardiac performance.

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Anti-Infective Action of an Anabolic Steroid. (23876)

M. GHIONE (Introduced by Jerome M. Glassman)

Farmitalia, Laboratori Ricerche Microbiologia e Chemioterapia, Milano

Taubenhaus(1-4) and others showed that formation of granulation tissue in animals and response to infection were stimulated by testosterone; and this seemed to be related to anabolic rather than androgenic action of testosterone. Moreover, Sala and Baldratti(5) screened a series of steroids synthesized in this laboratory(6) and showed that in castrated rats some testosterone analogs possessed potent effect on weight of levator ani muscle and weak androgenic activity as measured by change in ventral prostate gland.