As possible antimetabolites for calcium pantothenate, phenylalanine, cysteine, and folic acid, compounds 3, 4, 5, 6, and 11 were ineffective. Given intraperitoneally to Swiss mice having a *D. pneumoniae I* infection, compounds 2, 3, 4, and 6 were of no value. Compound 1 was ineffective as a possible spermatocidal agent. Also ineffective against a *Klebsiella pneumoniae* infection in Swiss mice given by various routes were compounds 1, 3, 4, 5, and 6. Compound 2, however, did show slight effectiveness.

REFERENCES

(1) Verderame, M., THIS JOURNAL, **50**, 312(1961).
(2) Foye, W. O., and Verderame, M., *ibid.*, **46**, 273(1957).
(3) Armstrong, M. D., and Lewis, J. D., *J. Org. Chem.*, **16**, 749(1951).
(4) Jacobs, W. A., and Heidelberger, M., *J. Am. Chem. Soc.*, **39**, 1455(1917).
(5) Goldberg, A. A., and Thomas, N. L., British pat. 664,363 (1952); through *Chem. Abstr.*, **47**, 613(1953).
(6) Harries, C., and Petersen, I., *Ber.*, **40**, 635(1910).
(7) Dahldom, R., and Ekstrand, T., *Acta Chem. Scand.*, **5** 107(1951).
(8) Finkelstein, J., *J. Am. Chem. Soc.*, **66**, 407(1944).

Effect of Phentolamine and Tolazoline on Some Metabolic Processes of Heart Homogenates

BY HARRY C. FROEDE and HAROLD C. HEIM

The addition of phentolamine enhances oxygen uptake of rat heart homogenates respiring in the presence of added succinate, but evokes an inhibitory effect with pyruvate as the substrate. These effects are not observed when phentolamine is replaced by tolazoline. Oxidation of α-ketoglutarate is not affected by either drug nor is the activity of a purified malic dehydrogenase. The addition of oxaloacetate to homogenates inhibits oxygen uptake and the inhibitory effect is not abolished by the prior addition of phentolamine. The total α-keto acid content of homogenates respiring in the presence of succinate was found to be somewhat higher than in those homogenates to which both succinate and phentolamine were added at the beginning of the experiments.

TOLAZOLINE and phentolamine are both 2-substituted imidazolines which evoke adrenergic blocking effects. It has been reported that the most pronounced side effects elicited by these drugs are related to a direct stimulation of the myocardium and include cardiac arrhythmias, anginal pain, tachycardia, and fibrillation (1–4).

A survey of the literature reveals that no studies have been undertaken to determine whether phentolamine or tolazoline affect metabolic processes of the heart, *in vitro*. Therefore, because of the widely held belief that drugs produce effects on tissues, at least in part, through an alteration of intracellular reactions, an investigation of the effects of these two drugs on oxidative processes of heart homogenates was conducted.

EXPERIMENTAL

Conventional manometric techniques were used to determine the effect of phentolamine and tolazoline on oxygen uptake in the presence of different added substrates including succinate, α-ketoglutarate, and pyruvate. Hearts from adult Sprague-Dawley rats of both sexes were used. The animals were stunned and decapitated, following which the hearts were immediately removed, cut open, and washed in cold 0.1*M* phosphate buffer. The ventricles were then dissected free, blotted dry on filter paper, weighed, and placed in a glass homogenizer immersed in ice and containing sufficient buffer so that 1.0 ml. of homogenate contained 50 mg., wet weight, of ventricle. The homogenates were centrifuged for 10 minutes at 480 × g in a Servall RC-2 centrifuge with an SS-34 rotor and at a temperature of 3°. The sediment was discarded and the supernatant was used as the homogenate. Ten animals were used in each experiment and each determination of oxygen uptake was performed in duplicate. No more than 10 minutes elapsed between the time the animals were killed and the preparation of the homogenates.

The concentrations of reagents added to the flasks are included in *Results*. The temperature of the bath was 37° and the gas phase was air. After a 10-minute equilibration period, the side arm contents were tipped into the vessels, the manometers closed, and readings taken at 15-minute intervals for a total of 90 minutes.

Keto-acid determinations were performed according to the method of Friedemann (5). The contents of the main compartments of two Warburg vessels were pooled and 2.0 ml. of the ensuing mixture was pipetted into 8.0 ml. of cold, 10% trichloroacetic acid solution. After centrifugation at 3000 × g for 15 minutes, 3.0 ml. of the clear supernatant

Received September 6, 1961, from the University of Colorado, School of Pharmacy, Boulder.
Accepted for publication October 6, 1961.
This investigation was supported by grant number H-5476 from the National Institutes of Health, United States Public Health Service.
The authors are most grateful for the generous amounts of phentolamine and tolazoline supplied by the Ciba Pharmaceutical Co.

was used for determination of total keto-acid content. The color which developed upon addition of 2,4-dinitrophenylhydrazine was measured at 435 mμ, with redistilled pyruvic acid being used as the standard keto-acid.

Malic dehydrogenase activity was determined according to the method of Ochoa (6). The oxidation of dihydro-β-diphosphopyridine nucleotide (DPNH) to diphosphopyridine nucleotide (DPN), with the concomitant reduction of oxaloacetate to malate, was measured by observing the decrease in absorbance (A_s) at 340 mμ with a Beckman model DU spectrophotometer. The enzyme concentration was adjusted so that 3.0 ml. of reaction mixture contained 40 units. The final concentration of DPNH and oxaloacetate were both 5×10^{-6} *M*. Malic dehydrogenase, DPNH, and oxaloacetate solutions were freshly prepared prior to each experiment.

RESULTS

Figure 1 shows that the oxygen consumption by rat heart homogenates in the presence of added succinate proceeds at a rapid rate for approximately 30 minutes, after which a decline in rate occurs. This is confirmatory to the results obtained by other investigators (7, 8). Figure 1 also shows, however, that if phentolamine is added at the start of the experiment in sufficient amount so that the final concentration is 7×10^{-4} *M*, oxygen consumption is much greater than in the absence of phentolamine. The rate is initially quite rapid and declines somewhat after about 30 minutes, but still remains greater than in the control flasks. The prior addition of tolazoline, so that final concentrations ranged between 1.4×10^{-2} *M* and 3.6×10^{-4} *M*, did not significantly alter the rate of oxygen uptake.

It has been established that oxaloacetate markedly inhibits the oxidation of succinate (9, 10) and it has been suggested that the decline in rate of oxygen uptake might be due to accumulation of oxaloacetate (7). Table I shows that the addition of oxaloacetate inhibited oxygen uptake in the presence of succinate by approximately 57% and that this inhibitory effect was not significantly altered by either phentolamine or tolazoline. In flasks containing pyruvate as the substrate, the addition of phentolamine, at final concentration of 7×10^{-4} *M*, caused a 70% inhibition of oxygen uptake, but this effect was not observed with tolazoline. When malate and succinate were both present, the oxygen uptake was somewhat less than that obtained when no malate was present, and the addition of phentolamine caused a 210% increase in oxygen consumption. Similar results were obtained with phentolamine in the experiments in which fumarate was present together with succinate. Tolazoline did not significantly alter oxygen consumption. When α-ketoglutarate was added as the substrate, the oxygen consumption was not changed by either phentolamine or tolazoline.

Analysis of flask contents at the end of the experiments revealed that total α-keto acids were present

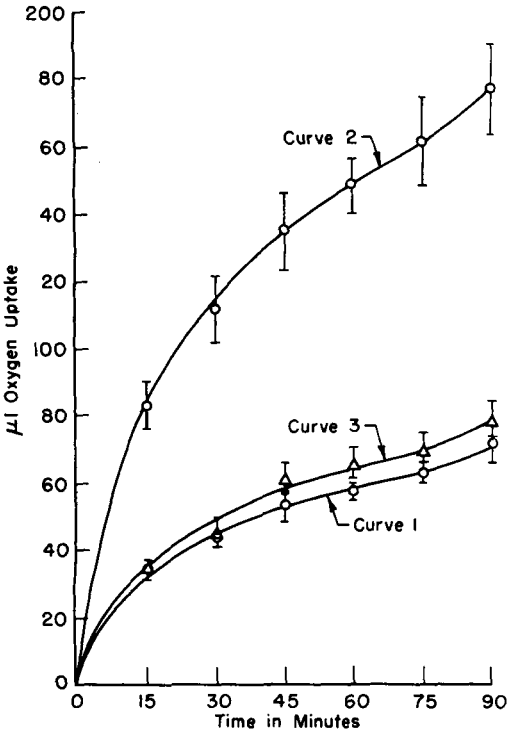

Fig. 1.—Effect of phentolamine and tolazoline on rate of oxygen uptake in the presence of added succinate. Curve 1, no drug; curve 2, phentolamine (7×10^{-4} *M*); curve 3, tolazoline (7×10^{-4} *M*). Experimental conditions are the same as shown in Table I.

TABLE I.—EFFECT OF PHENTOLAMINE AND TOLAZOLINE ON OXYGEN UPTAKE

Substrate	Drug	O_2 Uptake, mm.³/25 mg. Tissue/90 min.	Increase (+) or Decrease (−) Due to Drug, %
Succinate[a]	None	72 ± 5.7[g]	
	Phentolamine	176 ± 12.6	+140
	Tolazoline	77 ± 5.1	...
Pyruvate[b]	None	110 ± 7.9	
	Phentolamine	34 ± 6.0	−69
	Tolazoline	85 ± 9.7	−23
Succinate + malate[c]	None	54 ± 8.7	
	Phentolamine	165 ± 8.9	+210
	Tolazoline	58 ± 8.1	...
Succinate + fumarate[d]	None	41 ± 4.4	
	Phentolamine	132 ± 15.3	+280
	Tolazoline	47 ± 4.9	...
Succinate + oxaloacetate[e]	None	31 ± 5.5	
	Phentolamine	40 ± 7.7	...
	Tolazoline	34 ± 4.2	...
α-Keto-glutarate[f]	None	31 ± 4.4	
	Phentolamine	32 ± 4.1	...
	Tolazoline	31 ± 5.0	...

[a] Flasks contained 0.4 ml. 0.1 *M* succinate tipped from sidearm. [b] Flasks contained 0.4 ml. 0.1 *M* pyruvate containing 7.5×10^{-3} *M* ATP, 2.5×10^{-4} *M* DPN, and 2.5×10^{-3} *M* $MgCl_2$ added from sidearm. Main compartment contained 0.1 ml. 1.5×10^{-2} *M* nicotinamide. [c] Flasks contained 0.4 ml. 0.1 *M* succinate and 0.2 ml. 2×10^{-2} *M* malate, both added from sidearm. [d] Flasks contained 0.4 ml. 0.1 *M* succinate and 0.2 ml. 2×10^{-2} *M* fumarate, both added from sidearm. [e] Flasks contained 0.4 ml. 0.1 *M* succinate in sidearm, 0.1 ml. 10^{-2} *M* oxaloacetate in main compartment. [f] Flasks contained 0.4 ml. 0.1 *M* α-ketoglutarate and 0.1 ml. 10^{-3} *M* DPN, both added from sidearm. In addition, all flasks contained 0.5 ml. homogenate (25 mg. tissue), 0.3 ml. 5×10^{-4} *M* cytochrome C, 1.0 ml. 0.1 *M* phosphate buffer, pH 7.4 and water to make a total volume of 3.0 ml. Center wells contained 0.2 ml. 10% potassium hydroxide and filter paper strips. [g] Standard deviation = $\sqrt{\frac{\Sigma d^2}{n-1}}$.

TABLE II.—TOTAL KETO ACIDS IN FLASK CONTENTS AFTER 90 MINUTES

Substrate	Keto Acids, mcg./25 mg. Tissue[a]
Succinate	46.0 ± 2.2
Succinate + phentolamine	31.6 ± 3.7

[a] Results represent average of six determinations.

in greater amounts in flasks containing succinate than in those containing succinate plus phentolamine. These results are set forth in Table II.

Neither phentolamine nor tolazoline produced any significant effect on the activity of a purified malic dehydrogenase obtained from commercial sources.[1]

DISCUSSION

Although phentolamine and tolazoline are related chemically, tolazoline did not produce the *in vitro* metabolic effects observed with phentolamine. A slight inhibition of pyruvate oxidation was observed with tolazoline, but the effect was not nearly so marked as that observed with phentolamine.

Several investigators (7, 9, 10) have reported that very low concentrations of oxaloacetate strongly inhibit the oxidation of succinate. In this study it was found that the inhibitory effect of oxaloacetate could not be abolished by the prior addition of phentolamine. It has been suggested that oxaloacetate inhibits the oxidation of succinate through a competitive inhibition of succinic dehydrogenase (9–11) and at least one study has been conducted in which this phenomenon was observed with a purified succinic dehydrogenase preparation (12). It seems likely, however, that mass action effects might account for some of the inhibition when homogenates are used as the enzyme source because such preparations contain many enzymes other than succinic dehydrogenase and which could conceivably be affected by oxaloacetate. For example, the oxidation of succinate proceeds according to the pathway

$$\text{succinate} \xrightarrow{\text{DPN}} \text{fumarate} \rightarrow \text{malate} \xrightarrow{\text{DPN}} \text{oxaloacetate}.$$

The equilibrium constant (K') for the fumarate-malate system has been reported to be $8 \times 10^{-13}\ M$ (13). In view of the fact that the equilibrium lies far in the direction of malate, only minute amounts of oxaloacetate would be necessary to stop the conversion of malate to oxaloacetate.

The α-keto acid content, after 90 minutes incubation with succinate, was reduced by about 33% in those flasks to which phentolamine had been added at the beginning of the experiment. Wolff and Ball (7), in their studies on the effect of thyroxine on succinate metabolism, found that thyroxine inhibited malic dehydrogenase and concluded that thyroxine prevented the accumulation of oxaloacetate. The concentration of succinate used in these studies was much greater than that used by Wolff and Ball. Thus, if oxaloacetate is a competitive inhibitor of succinic dehydrogenase, a greater quantity would be required to inhibit oxygen uptake in the experiments reported herein. It was found, however, that the total amount of α-keto acids which accumulated in the flask contents is in close agreement with the values reported by Wolff and Ball. Furthermore, the rate of oxygen uptake during the first 15 minutes was about 140% greater in the flasks containing phentolamine, whereas thyroxine has been reported to produce no change in the initial rate (7). It would not appear conceivable, therefore, that phentolamine increases the oxygen uptake in the presence of added succinate by preventing the accumulation of oxaloacetate.

Close correlation cannot as yet be drawn between the effects observed *in vitro* and the response elicited by drugs *in vivo*. It has been established that even slight alterations in the structural configuration of many drugs produce marked changes in pharmacological activity. Although phentolamine and tolazoline are both imidazoline derivatives, they differ structurally to a considerable extent. This structural difference could possibly be related to the different effects produced by the two drugs on the *in vitro* systems used in this study and to the effects produced on the heart, *in vivo*.

It should be stressed that, with the exception of the experiments involving purified malic dehydrogenase, this study was conducted with homogenates as the enzyme source. In such preparations there are many reactions occurring which evoke indirect, rather than direct, effects on the enzymatic reactions of the citric acid cycle. In describing the effects of a drug on aerobic processes one cannot neglect the possibility that the drug might alter reactions which are indirectly involved with the cycle and which, therefore, escape observation when studies are performed using citric acid cycle intermediates as substrates. It is, therefore, not possible to elaborate a mechanism of action of phentolamine on the basis of the effects produced by this drug on enzymes of the citric acid cycle, *in vitro*. However, since phentolamine inhibits oxygen uptake with pyruvate as substrate, enhances oxygen uptake in the presence of succinate, evokes no effect on α-ketoglutarate oxidation, and has no effect on malic dehydrogenase, it could conceivably act at the level of the electron transport system or the accompanying phosphorylation system.

[1] Sigma Chemical Corp., St. Louis, Mo.

REFERENCES

(1) Goodman, L. S., and Gilman, A., "Pharmacological Basis of Therapeutics," 2nd ed., McMillan Co., New York, N. Y., 1955, p. 585.
(2) Marriott, H. J., *Ann. Internal Med.*, **46**, 1001(1957).
(3) Davidson, J. D., *J. Am. Med. Assoc.*, **162**, 108(1956).
(4) Emanuel, D. A., *et al.*, *ibid.*, **161**, 436(1956).
(5) Friedemann, T. E., "Methods in Enzymology," Vol. 3, Academic Press, New York, N. Y., 1957, p. 414.
(6) Ochoa, S., "Methods in Enzymology," Vol. 1, Academic Press, New York, N. Y., 1955, p. 735.
(7) Wolff, E. C., and Ball, E. G., *J. Biol. Chem.*, **224**, 1083 (1957).
(8) Deitrich, R. A., and Heim, H. C., THIS JOURNAL, **45**, 562(1956).
(9) Das, N. B., *Biochem. J.*, **31**, 1124(1937).
(10) Pardee, A. B., and Potter, V. R., *J. Biol. Chem.*, **176**, 1085(1948).
(11) Keilin, D., and Hartree, E. F., *Proc. Roy. Soc. London*, **B-129**, 277(1940).
(12) Kearney, E. B., and Singer, T. P., *J. Biol. Chem.*, **219**, 963(1956).
(13) Burton, K., and Wilson, T. N., *Biochem. J.*, **54**, 86 (1953).