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Augmentation of Oxygen Consumption of Rat Brain *in vitro* by Various Carbohydrate Intermediates. (27709)

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When during other studies it was unexpectedly discovered that addition of citrate ion to rat brain slices *in vitro* produced as much as a 27% increase in their rate of oxygen consumption, no previous report of a similar phenomenon could be found except that of Krebs and Johnson(1) who noted that citrate increased the respiratory rate of pigeon breast muscle *in vitro*. Lipsett and Crescitelli(2) found that citrate prevented the increase in oxygen consumption obtained by adding 0.1 M KCl to rat brain slices in a dextrose substrate. Therefore the above findings on rat brain were investigated *in vitro* further; this paper concerns preliminary results showing some of the interrelated effects of ions and various substrates.

Approach and methods. The standard technic of the Warburg method was used. Time elapsing between decapitation of rats and beginning of equilibration was 25 minutes (± 5) for both brain slices and brei. Concentrations of constituents in the flasks and the errors of the differences of the means are given in the tables. The pH at the start was 6.8-7.0 and changed about 0.4 unit during experimental runs. The lack of effect of a nonmetabolized carbohydrate, mannitol, was shown by the finding that oxygen consumption (μl per mg fresh wt/hr) of 0.0166 M dextrose alone was 1.26 while that of 0.0166 M dextrose plus 0.0416 M mannitol was 1.29.

Results. In the first experiment (Table I, A) the effect of citrate at .0416 molar concentration in the presence of only one substrate, dextrose, was a considerable enhancement of O_2 consumption of brain slices and brei (27% and 51%, respectively). Optimal concentration values of dextrose alone and of combinations of dextrose and citrate are shown in Table I (B and C). Similar enhancement was also shown clearly for the brains of several species, mouse, cat, rabbit, guinea pig and dog, at optimal concentrations found for the rat (Table II). When 3 rat organs other than brain were tested, enhancement was found in heart and kidney, but not in liver (Table II).

We investigated how much increase in O_2 consumption resulted from addition of Krebs-cycle intermediates with and without dextrose (Fig. 1, 2). In Krebs-Ringer phosphate medium only lactate gave a greater respiratory rate than dextrose alone, while pyruvate, oxalacetate, and ketoglutarate gave the same, and malate, fumarate, and citrate gave less (Fig. 1C). In the presence of dextrose, however, only citrate gave enhancement (Fig. 2B) in Krebs-Ringer medium.

If phosphate ion concentration was varied, the substrate effects were different. When

each substrate was used alone, phosphate ion was shown to increase respiratory rate with some (dextrose and lactate) and to decrease it with others (fumarate, malate, ketoglutarate, and citrate) (Fig. 1 D and E). Two substrates showed no change (pyruvate and oxalacetate).

In the presence of 2 substrates, *i.e.*, dextrose and one intermediate, the singular enhancement of respiratory rate by citrate ion,

TABLE I. Oxygen Consumption* of Rat Brain Slices and Brei in Varying Concentrations of Dextrose and Citrate Ion.

Molarity† dextrose	Molarity citrate	Brain slices	Brain brei
		A	
.0166	.01	1.13 (2)‡ ± .11§	.81 (5) ± .25
.0166	.00416	1.24 (4) ± .08	.87 (5) ± .09
.0166	.0083	1.10 (4) ± .04	1.10 (5) ± .27
.0166	.075	1.33 (4) ± .10	.86 (5) ± .09
.0166	.0166	1.30 (4) ± .01	1.08 (5) ± .15
.0166	.0416	1.70 (2) ± .04	1.46 (5) ± .07
.0166	.083	1.65 (2) ± .05	1.31 (5) ± .20
		B	
.0022	0	1.16 (6) ± .11	.88 (6) ± .05
.00416	0	1.37 (55) ± .11	1.04 (10) ± .18
.0166	0	1.23 (10) ± .08	.80 (4) ± .15
.0416	0	1.28 (7) ± .09	
.0830	0	1.17 (2) ± .05	
.166	0	1.03 (2) ± .02	
		C	
.00416	.0083	1.52 (3) ± .17	
.00416	.0166	1.42 (3) ± .14	
.00416	.0416	1.74 (9) ± .14	1.57 (21) ± .26
.0022	.0416	1.35 (5) ± .10	1.60 (5) ± .09
.0083	.0416		1.26 (5) ± .07
0	.0416	1.01 (6) ± .12	.69 (6) ± .09
0	.0830	1.14 (2) ± .16	

* Microliter/mg fresh wt/hr.
† Final molar concentration in Krebs-Ringer phosphate buffer in reaction vessels.
‡ ( ) indicates number of determinations.
§ Standard deviation from mean.

TABLE II. Oxygen Consumption* of Brain from Six Animal Species and of Rat Organs with Dextrose or Dextrose and Citrate as Substrate.

Animal species brain	Substrate† .00416 dextrose .0416 citrate	Substrate† .00416 dextrose	% increase with citrate
Rat slices	1.74 (9)‡ ± .14§	1.37 (55) ± .11	27
" brei	1.57 (21) ± .26	1.04 (10) ± .08	51
Guinea pig slices	1.27 (6) ± .16	.97 (4) ± .15	31
" " brei	1.01 (4) ± .04	.58 (4) ± .05	74
Mouse slices	1.99 (2) ± .06	1.79 (2) ± .09	11
" brei	1.82 (3) ± .06	1.27 (4) ± .20	43
Cat slices	1.21 (4) ± .09	.86 (4) ± .05	41
Rabbit slices	1.13 (4) ± .08	.74 (4) ± .02	52
" brei	1.01 (4) ± .12	.40 (4) ± .06	150
Dog slices	.90 (2) ± .01	.70 (2) ± .19	28
" brei	.86 (2) ± .06	.50 (2) ± .07	72
Rat tissue			
Kidney slices	2.20 (7) ± .11	1.90 (5) ± .07	15
Liver "	.98 (5) ± .06	.98 (2) ± .01	0
Heart "	.82 (5) ± .09	.61 (4) ± .04	34

See footnotes Table I.

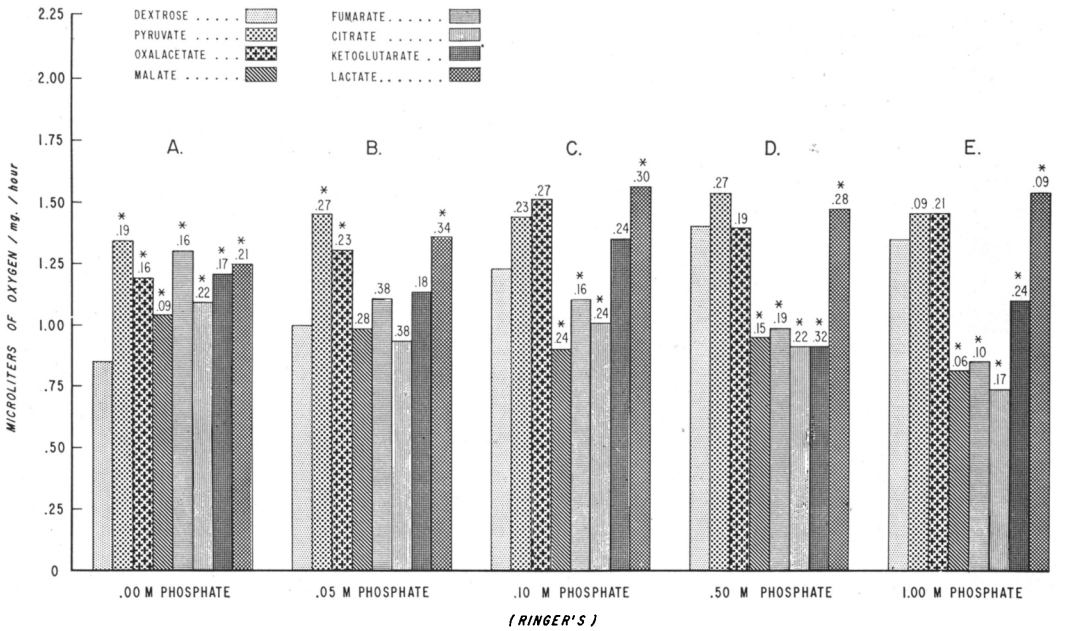

FIG. 1. Oxygen consumption of rat brain slices in 0.0416 M Krebs cycle intermediates. In this and the other figures the standard error of the differences between the means was calculated. To determine significant comparisons between the effect of various substrates and dextrose alone under given conditions, the appropriate difference of the means was multiplied by the Student "t" at the 5% level of significance. Significant differences were then determined from the statistical tables and are indicated by a star at the top of the corresponding bar in the figures.

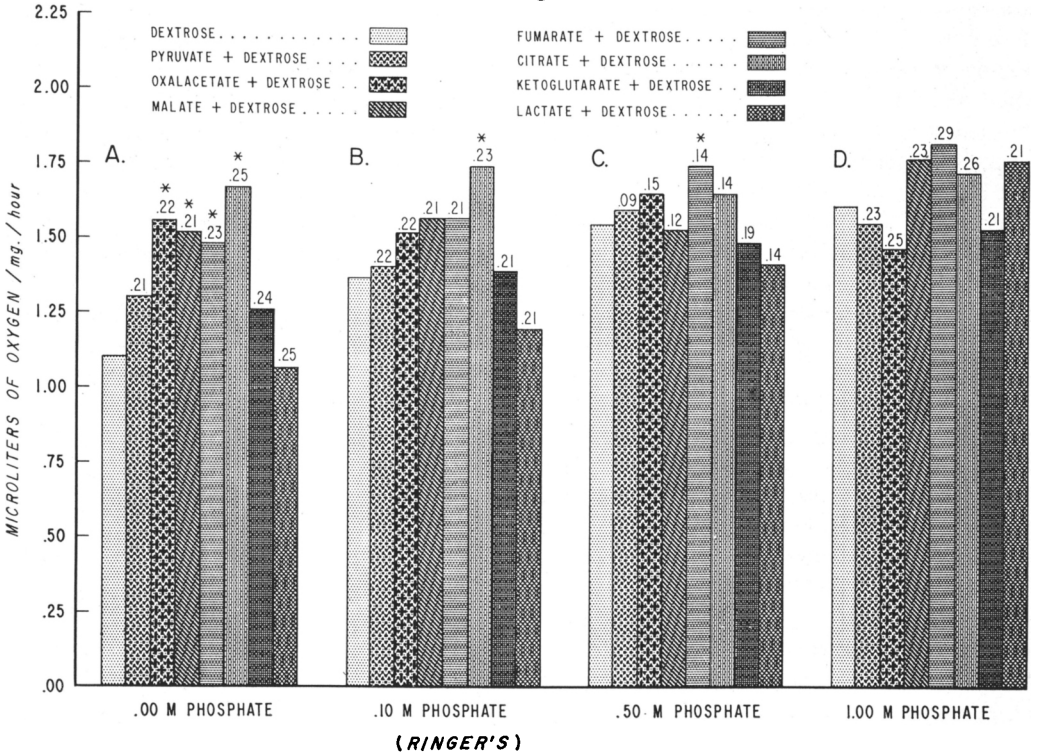

FIG. 2. Oxygen consumption of rat brain slices in .0166 M dextrose alone and together with .0416 M Krebs cycle intermediates in various phosphate ion concentration.

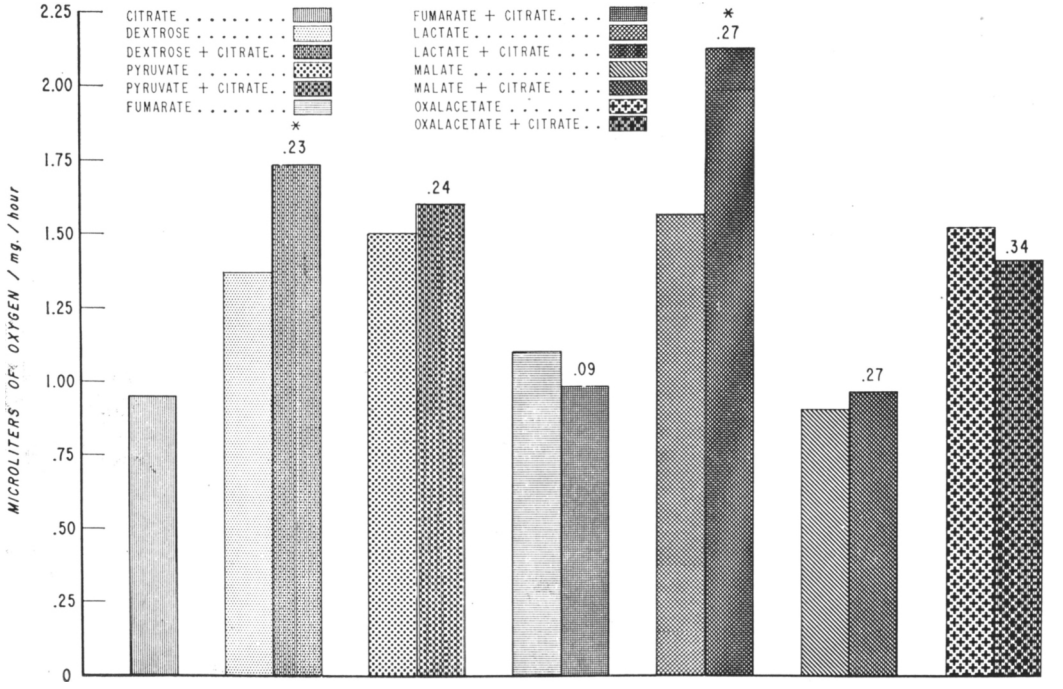

FIG. 3. Oxygen consumption of rat brain slices in .0416 M Krebs cycle intermediates with and without citrate ion (.0416 M) in Krebs-Ringer phosphate.

shown above, was found to be dependent upon the presence of phosphate ions. Thus decreasing concentrations of phosphate permitted more and more enhancement by other intermediates, (Fig. 2A). Concentration of phosphate salts was varied without replacement by other ions since phosphate salts contribute only 11% of the total molarity of Krebs-Ringer phosphate buffer.

If dextrose was replaced by another substrate, citrate enhancement was found only for lactate (Fig. 3), wherein a 27% increase was measured.

Summary. (1) Citrate ion in a concentration of 0.0416 M enhanced oxygen consumption of rat, cat, dog, rabbit, guinea pig, and mouse brain slices and brei in dextrose and Krebs-Ringer phosphate buffer. Optimal concentration for dextrose was 0.0166 M. This effect was noted also in rat heart and kidney. (2) Of the 7 Krebs-cycle intermediates used as substrates individually, only lactate ion in Krebs-Ringer phosphate maintained respiration of rat brain better than did dextrose. (3) When the possibility of enhancement by substrates other than citrate was tested, no other ions reacted in this way in Krebs-Ringer phosphate. (4) Phosphate ion concentration played an important role; very different results were found in its absence. (5) The citrate ion enhanced the respiratory rate of rat brain not only in the presence of dextrose but also in the presence of lactate. (6) It is noteworthy that the respiratory rate of brain *in vitro* is affected in a unique fashion by citrate ion; that lactate is used by brain in the absence of dextrose more effectively than any other substance tested; and that phosphate ion influences the effects of various substrates.

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