Table 1. ΔG_t^0 values in Jmoles^{-1}

t (°C)	H^+Cl^-	H^+Br^-	H^+I^-	H^+SCN^-
10% dioxane				
15	455	200	− 531	− 357
25	420	165	− 560	− 338
35	1,770	306	− 511	− 357
45	3,110	415	− 724	− 386
20% dioxane				
15	928	252	− 878	− 347
25	1,009	230	− 897	− 270
35	1,103	328	− 965	− 299
45	1,205	577	− 1,148	− 270
30% dioxane				
15	-1,543	520	− 1,187	− 482
25	900	734	− 1,023	− 492
35	2,490	835	− 1,399	− 550
45	3,740	1,124	− 1,640	− 656
40% dioxane				
15	2,518	808	− 1,409	− 589
25	3,330	1,271	− 1,245	− 608
35	4,600	1,687	− 1,592	− 608
45	5,870	2,076	− 2,055	− 647

Table 2. Ionic ΔG_t^0 values in J

t (°C)	H^+	Cl^-	Br^-	I^-	SCN^-
10% dioxane					
115	− 5,540	7,310	5,846	5,029	5,183
25	− 5,500	5,920	5,665	4,940	5,162
35	− 11,800	13,570	12,106	11,289	11,443
45	− 19,800	22,910	20,215	19,076	19,414
20% dioxane					
15	− 28,800	29,903	29,128	27,835	28,453
25	− 10,400	11,409	10,630	9,503	10,130
35	− 11,300	12,403	11,628	10,335	11,001
45	− 12,900	14,105	13,477	11,752	12,630
30% dioxane					
15	− 14,800	17,290	15,635	13,401	14,318
25	− 10,600	11,500	11,334	9,577	10,108
35	− 20,800	23,290	21,635	19,401	20,250
45	− 28,400	32,140	29,524	16,760	27,744
40% dioxane					
15	− 21,000	25,600	22,687	19,408	20,411
25	− 24,000	27,330	25,271	22,755	23,392
35	− 32,400	37,000	34,087	30,808	31,792
45	− 41,500	47,370	43,576	39,445	40,853

acids against the anionic radius (r_-) according to the following equation[7]:

$$\Delta G_t^0 = \Delta G_t^0(H^+) + Kr_-^{-1}, \quad (1)$$

where K is a constant.

Since the ΔG_t^0 terms for ions in an electrolyte are additive[13], the ionic ΔG_t^0 values for Cl^-, Br^-, I^- and SCN^- were calculated from the ΔG_t^0 values for H^+Cl^-, H^+Br^-, H^+I^- and H^+SCN^-, respectively. The ionic ΔG_t^0 values are presented in table 2. The $\Delta G_t^0(H^+)$ values are negative at all the temperatures and solvent mixtures studied. So dioxane-water mixtures are more basic than water and the basicity of dioxane-water mixtures is enhanced with the increasing proportion of dioxane in the mixture.

All the 4 anions studied here are found to be in higher free energy state in dioxane-water mixtures and their order is $Cl^- > Br^- > SCN^- > I^-$. Considering the 3 halides, it is seen that the free energy of transfer decreases with increasing ionic diameter. Since the ΔG_t^0 values for SCN^- lies in between Br^- and I^- it can be concluded that the ionic diameter of thiocyanate ion lies in between Br^- and I^-. It is also seen that the ΔG_t^0 values for H^+SCN^- fit well on the straight line graph ΔG_t^0 against r_-^{-1} according to equation (1) if the ionic radius of SCN^- is taken to be equal to 2.08 Å which is in between r_{Br^-} (1.951 Å) and r_{I^-} (2.168 Å)[14].

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Development of oxidative metabolism in the non-innervated optic lobe of the chick

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Summary. Early removal of the optic cup of the chick embryo prevents innervation of the contralateral optic lobe. This reduces the rate of development of citrate synthetase. The posthatch increase of the level of this enzyme related to oxidative metabolism is not impaired by denervation of the chick optic lobe.

During cerebral maturation, there is a major change from predominantly glycolytic metabolism to an intense oxidative metabolism. This shift occurs after the cessation of neurogenesis and prior to the onset of neuronal excitability[1]. Activity of the citric acid cycle may be necessary for several specific features of neuronal metabolism. These include the high energy requirements of the sodium pump and of axoplasmic transport[2]. Furthermore, operation of the citric acid cycle is needed for the production of several amino acids found in high concentration in the mature

brain, including glutamate, glutamine, asparate and gamma-aminobutyrate. The adult brain has an absolute and continuous dependence on a high rate of oxidative metabolism. We have studied the extent to which the development of an enzyme related to aerobic metabolism (citrate synthetase) is impaired after prevention of innervation from the periphery of the embryonic chick optic lobe. The effect of denervation of the more mature tectum of the new hatch chick has also been measured.

Methods and materials. In order to prevent innervation of the embryonic optic lobe, chick eggs of a white leghorn strain were maintained at 38 °C for 48 h in a rotating incubator, after which they were incubated in an upright position (with the blunt pole upward) for a further 24 h. A hole around 1 cm in diameter was then cut over the air space and the embryo was exposed by peeling back the allantoic membrane. The right eye, which almost invariably faced upward, was electrocoagulated using a sharpened bipolar forceps insulated except at the tip, attached to a Malis Bipolar Coagulator (Codman Inc., Randolph, Mass.). The egg was then resealed with a circle of paraffin wax (m.p. 56 °C) upon which a flame-heated coverslip was placed. The remainder of the incubation was carried out with the egg in a vertical position. To study the effects of deafferentation of the functioning optic lobe, eyes were removed from new hatched chicks under light chloroform anesthesia.

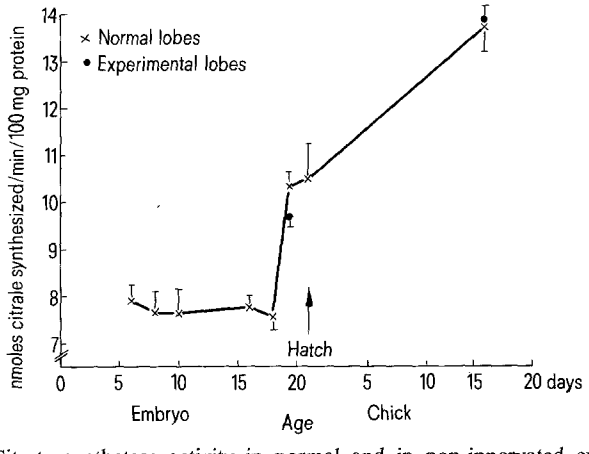
Citrate synthetase was assayed by the method of Srere[3]. Briefly, 10% homogenates of tissues were prepared in 175 mM KCl, 10 mM glutathione and 2 mM EDTA, pH 7.0, and frozen at −70 °C. Thawed homogenates were diluted tenfold in 10 mM tris-HCl, pH 8.0. The rate of synthesis of citrate from acetyl CoA and oxalacetate at 22 °C was estimated by the concurrent production of coenzyme A which reacts with dithio-bis-2-nitrobenzoic acid to form the mercaptide ion which has an absorbance maximum at 412 nm. Under the conditions used, the enzyme reaction was linear for over 15 min. Nonenzymic background formation of CoA was very low and was corrected for. Protein was determined by the method of Lowry et al.[4] and results were expressed as nmoles citrate formed/100 mg protein/min. Each data point presented was derived from the mean of at least 6 optic lobes.

Results and discussion. The rate of citrate formation at 22 °C was linear throughout the 15-min incubation period. Since doubling the homogenate concentration in the incubation mixture, caused a precisely twofold increase in the rate of color development, the enzyme and not the substrate was limiting in this assay.

Citrate synthetase levels remained constant for the first 18 days of incubation of normal chick embryos (figure). After this time there was an abrupt increase in the amount of enzyme present when expressed per unit protein. After hatch, citrate synthetase content continued to increase at a slower rate. Thus this enzyme develops at the same rate as succinic oxidase[5]. Since the protein concentration of the chick brain relative to wet weight almost doubles between day 8 of incubation and hatch[5] the rise of this enzyme would appear more gradual if it were expressed on a wet weight basis. However, expression of enzyme levels relative to protein best demonstrates the sudden increase in emphasis given to the production of this enzyme by the protein synthetic machinery.

Early removal of the optic cup caused a significant reduction ($p < 0.05$, Student's 2-tailed t-test) in the specific activity of citrate synthetase in the non-innervated optic lobes relative to partner control lobes, on day 19 of incubation, immediately prior to hatch. Due to the high mortality rate of operated embryos on the day before hatch, it was not possible to determine accurately the enzyme levels of 20-day-old embryos. The increase in enzyme activity of the experimental lobe was only 75% of the normal (figure). The significance of this difference is accentuated by the finding that in all cases (11 embryos), the level of citrate synthetase was higher in the control lobe than in the experimental lobe of the same bird. Control lobes contralateral to the remaining eye of operated birds had enzyme levels indistinguishable from those of unoperated chicks of the same age. The value for the lobes from unoperated chicks of the same age. The value for the lobes from unoperated birds was 10.35 nmoles citrate synthesized/min/100 mg protein, with a SE of ±0.31 while the corresponding value for control lobes from experimental birds was 10.41±0.33. Thus failure of the optic lobe to receive its unusual input from the eye can selectively retard the maturation of a key enzyme related to oxidative metabolism.

Enzyme levels were measured in experimental and control lobes of 15-day-old birds which previously had a single eye removed within 12 h after hatch. 15 days after enucleation of new hatched chicks the specific activity of control and denervated optic lobes was identical although the deafferented lobe is much smaller and has a lower content of protein and RNA[6]. The value nmoles citrate synthesized/min/100 mg protein) for experimental lobes was 13.8±0.2 and for control lobes was 13.5±0.4. These are both very similar to the level of citrate synthetase of optic lobes of the unoperated, normal 15-day-old bird (13.7±0.4) (figure). Thus removal of the afferent supply after it has been established, does not exert a specific effect on the concentration of this enzyme. Non-innervation of the chick tectum has also been found to exert a much more drastic effect than post hatch denervation on the expression of neurotransmission related function[7].



Citrate synthetase activity in normal and in non-innervated experimental optic lobes of the developing chick embryo and young chick. Each point represents the mean of values derived from 6 to 14 lobes. Bars indicate SEM.

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