# The Activities of NADP Transhydrogenase and Cytochrome c Reductases in Developing Chick Embryos, Especially at Hatching

A mitochondrial enzyme, NADP transhydrogenase (EC 1.6.1.1) (TH), has been found in organs of various animals including chicks[1,2]. This enzyme, which plays a regulating role in $NADPH_2$ oxidation in rat[3], has recently been considered in the electron transport system of early chick embryos[4]. In bovine heart thyroxine is known to inhibit this enzyme directly[5]. To learn its significance during development in pectoral and cardiac muscle of embryonic and newly hatched chicks, TH activity, together with that of $NADPH_2$ cytochrome c reductase (EC 1.6.2.3) (TCR) and of $NADH_2$ cytochrome c reductase (EC 1.6.2.1) (DCR), has been studied.

To obtain the cellular fraction used, tissue homogenate in 0.25 *M* sucrose solution was centrifuged twice, each for 10 min at 700 *g*, and by further centrifugation of the combined supernatant the fraction sedimenting between 3000 and 15,000 *g* was obtained and washed. The final precipitate was suspended in 1 ml of 0.12 *M* KCl solution[6]. Preliminary experiment showed that in chicks of various ages this fraction is the most suitable in studying all 3 enzymes examined. In assaying TH[2], TCR[7], and DCR[8], the increase in absorbance at 340 or 550 nm at room temperature was measured spectrophotometrically. Protein was estimated at 280 nm.

During 13–18 days of incubation TH activity in pectoral muscle is relatively steady (Figure 1). At 19 days, however, the activity is nearly double the level of the previous day, then reaches the maximum at 20 days (6–12 h before hatching). The newly hatched chicks maintain this high activity up to 36–40 h after hatching. In contrast, the activity of TCR (Figure 1) closely reciprocates that of TH (Table), by dropping abruptly 37% at 19 days and remaining at this low level until further reduction occurs at 2 days of age. The DCR level shows an 18% reduction during the 2 days previous to hatching. In cardiac muscle (Figure 2) the level of the TH activity throughout is almost the same as in pectoral muscle. Again, there is a clear reciprocal relationship between

The activities of transhydrogenase expressed as a ratio to the other enzymes

| Age (days) | TH/TCR Pectoral M. | Cardiac M. | TH/DCR Pectoral M. | Cardiac M. |
|---|---|---|---|---|
| 13–17 | 0.12–0.30 | 0.17–0.29 | 0.02–0.042 | 0.018–0.028 |
| 18 | 0.33 | 0.30 | 0.040 | 0.029 |
| 19 | 0.96 | 0.73 | 0.099 | 0.059 |
| 20 | 0.91 | 0.89 | 0.105 | 0.072 |
| Hatching | | | | |
| 1 | 0.94 | 0.60 | 0.78 | 0.058 |
| 2 | 1.27 | 0.46 | 0.81 | 0.051 |


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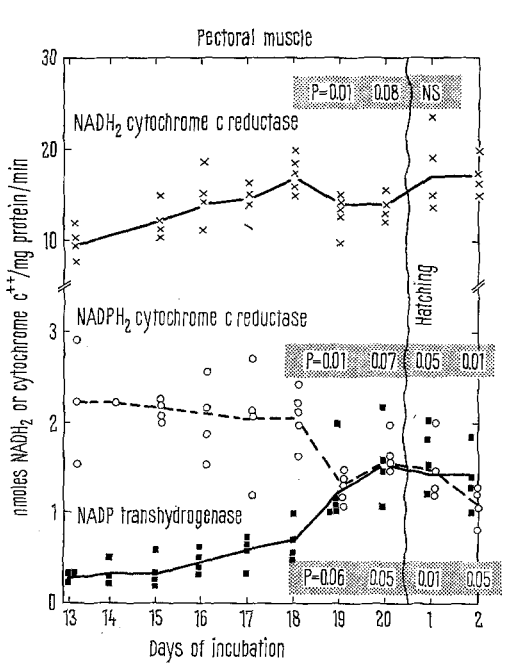


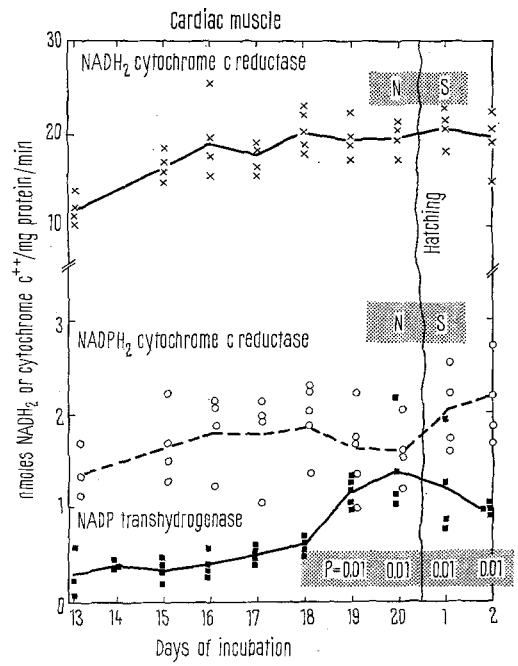


Figs. 1 and 2. The individual and average values of enzyme activities in pectoral (Figure 1) and cardiac (Figure 2) muscles of chicken. The shaded areas give the *P* value for each day's activity compared with that of 18 days for statistical significance (*P* of 0.05 or less). NS denotes $P > 0.1$.

TH and TCR (Table), although the initial low activity of TCR at 13–15 days and the lack of statistical significance in changes at later days make the relationship less definite. DCR in cardiac muscle remains static.

The main finding in this experiment is the maintenance of a reciprocal relationship between TH and TCR throughout, especially the correspondence of the sudden TH increase to the sudden TCR decrease before hatching. In rat kidney, liver, and brain a greater TH activity correlates with a greater phosphorylation quotient, indicating a role of TH in regulating oxidative phosphorylation associated with $NADPH_2$[3]. The oxidation of $NADPH_2$ by pathways other than TH indicates reductive biosyntheses[9,10] rather than a high-energy phosphate yield[11]. Therefore, our results could indicate a pre-hatching conversion from the TCR to the DCR route for a greater efficiency of energy production.

Previous reports are in accord with the above view. A significant increase of NADP-specific isocitrate dehydrogenase (ICDH) occurs in chick heart mitochondria between 18 days and the day after hatching[10], the period coinciding with TH increase in this experiment. In rodent heart mitochondria ICDH requires TH to complete its oxidative pathway[12], and in kidney, liver, and brain the activity of ICDH also varies proportionately with TH activity[3]. Furthermore, chick liver ICDH increases immediately before hatching, reaches a peak at hatching, and falls to half that level in 12 days; however, its peak level can be maintained and even increased by starving the chicks[13], which again relates these enzyme levels to energy requirement. In chick pectoral muscle the M isozyme of lactic dehydrogenase increases just before hatching[14].

The initially high level of TCR in pectoral muscle could mean a greater relative importance of the pentose cycle in early chick embryos[15]. It appears that the changes of TH and TCR activities are too small a proportion to be reflected upon the activity of DCR, to which many other oxidative enzymes are closely related. In this experiment there is no essential difference between pectoral and cardiac muscle.

*Résumé.* Dans les muscles pectoraux et cardiaque de l'embryon de poulet, une relation réciproque persiste entre transhydrogénase NADP et cytochrome c reductase $NADPH_2$, surtout à la période qui précède immédiatement l'éclosion. Cette relation exprime vraisemblablement l'accomplissement d'une plus grande demande d'énergie.

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## Metabolism of Metopirone and 3-(1,2,3,4-Tetrahydro-1-oxo-2 naphthyl)-pyridine in Relation to DMBA Induced Adrenal Necrosis

The polycyclic aromatic hydrocarbon 7,12-dimethylbenz(a)anthracene (DMBA), a potent carcinogen, also causes adrenal necrosis in rats[1]. Present evidence[2] indicates that the proximal adrenonecrotic agent is 7-hydroxymethyl-12-methylbenz(a)anthracene (7-OHM-12-MBA), a metabolite of DMBA produced by the liver[3].

One method of preventing adrenal damage by DMBA is by the administration of 2-methyl-1, 2-bis-(3-pyridyl)-1-propanone (metopirone, Ciba Su-4885) or 3-(1, 2, 3, 4-tetrahydro-1-oxo-2 naphthyl)-pyridine (Ciba Su-9055). It has been suggested[4] that the protective effect of these compounds resides in the steric similarity between the reduced form of metopirone, a known metabolite[5], and 7-OHM-12-MBA, resulting in competition for adrenal receptor sites. It was therefore considered of interest to synthetize reduced metopirone and Su-9055 in order to test their ability to protect rats against DMBA-induced adrenal necrosis, and to try and correlate any effect of these drugs on DMBA metabolism with their protective action. The metabolism of Su-9055 by rat liver in vitro was also investigated.

*Methods.* Both reduced metopirone and Su-9055 were obtained in good yield by the method of KRAULIS et al.[5] using sodium borohydride. Reduced Su-9055 was recrystallized from petroleum ether-acetone as white crystals, mp 152-155 °C with absorption maxima at 220, 256, 262 and 268 nm and a molar extinction coefficient of 5400 at 262 nm.

DMBA (30 mg in 1.5 ml sesame oil) was administered by stomach tube to young female Sprague Dawley rats (140–180 g) and the Su-compounds (50 mg/ml oil) were injected i.p. 2 h before the polycyclic hydrocarbon. Ethionine dissolved in 0.9% saline at 25 mg/ml was given i.p. according to the schedule of WHEATLEY[6]. Generally, one group of rats was killed 3 days after DMBA administration and the adrenals examined for gross hemorrhage and necrosis while 2 other groups were killed 4 and 18 h after treatment with protective compounds and a microsomal (8000 *g* supernatant) fraction of the liver prepared for biochemical studies.

Metopirone, Su-9055 (0.2 mg) or 7,12-dimethylbenzanthracene-12-$^{14}$C (0.051 μc in 2 μg) was added to the tissue preparations obtained from 50 mg liver and incubated in 4 ml 0.1 *M* potassium phosphate, pH 7.4 for 1 h under $O_2$ together with reduced nicotinamide nucleotides (0.3 m*M*) as described previously[7]. After extraction with $CH_2Cl_2$ the products formed from the Su-compounds were examined by paper chromatography in toluene

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