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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Table I. Incidence of DMBA-induced adrenal necrosis in rats treated with Su-compounds

Group	Pretreatment[a]	Dose (mg)	No. of rats with adrenal necrosis after treatment with DMBA
I	Oil control	–	8/8
II	Su-9055	30	0/4
		15	2/5
		10	4/4
III	Reduced Su-9055	15	1/4
		10	4/5
		6	5/5
IV	Su-4885	30	0/4
		15	2/5
V	Reduced Su-4885	15	1/3
		6	3/4
		3	2/2

[a] The Su-compounds were injected i.p. 2 h before oral DMBA (30 mg). Other experimental conditions as described in text.

Recently, WHEATLEY[10] found that treatment of rats with ethionine before injection of the Su-compounds abolished their protective action against DMBA and he therefore suggested that metopirone and Su-9055 may be acting by stimulating the formation of drug-metabolizing enzymes in the liver. Since our previous results[4] did not support this hypothesis, it was decided to repeat these experiments using groups of 3 rats which were treated as shown in Table II and as discussed under methods. Any alteration in DMBA metabolism could thus be compared directly with the occurrence of necrosis. Our results confirmed the biological findings of WHEATLEY but there was no change in the metabolism of ^{14}C-DMBA after 4 h and only a relatively small increase in the formation of water-soluble products 18 h after treatment with the Su-compounds. Yet, under similar conditions, the polycyclic hydrocarbon, 3-methylcholanthrene, produced a marked increase in the metabolism of DMBA. Since both the Su-compounds and MC protected the animals against adrenal necrosis, the induction or activation of DMBA-metabolizing enzymes by metopirone and Su-9055 is unlikely to account for this effect. This however does not exclude stimulation of the synthesis of some other active protein species which in some way interferes with the sequence of biochemical events culminating in adrenal necrosis[11].

Table II. Effect of ethionine on the action of Su-compounds in vivo in relation to the metabolism of ^{14}C-DMBA by rat liver microsomes

Group	Treatment schedule[a] (% of added ^{14}C in aqueous fraction after ether extraction)						Adrenal necrosis
	− 6 h	− 4 h	0 h	+ 2 h	after 4 h	after 18 h	
I	Saline	oil	DMBA	Saline	18.2	20.8	+
II	Ethionine	oil	DMBA	Ethionine	21.7	18.2	+
III	Saline	Su-4885	DMBA	Saline	20.9	27.6	–
IV	Ethionine	Su-4885	DMBA	Ethionine	19.0	16.7	±
V	Saline	Su-9055	DMBA	Saline	18.8	–	–
VI	Ethionine	Su-9055	DMBA	Ethionine	–	–	±
VII	MC[b]	–	DMBA	–	–	52.3	–

[a] Weights administered at time intervals indicated. DMBA, 30 mg; Su-compounds, 30 mg; ethionine, 50 mg at − 6 h and 25 mg at + 2 h. [b] Methylcholanthrene (MC), 10 mg in 1 ml oil was given orally 18 h previously. Rats used for biochemical studies were killed 4 h and 18 h after administration of protective compounds. Condition of incubation as described in the text.

saturated with propylene glycol[8] and also by TLC in ethyl acetate, benzene, methanol (10:2:0.75)[5]. Any UV-absorbing areas were eluted with ethanol for spectrum determination and the chromatograms visualized by exposure to iodine vapour. Ether-soluble ^{14}C-DMBA metabolites were located by autoradiography after chromatography in benzene-ethanol (19:1)[3] and quantitative data obtained as described previously[7].

Results and discussions. The results in Table I indicate that comparable doses of the original and reduced forms of metopirone and Su-9055 have about the same effect in preventing adrenal necrosis.

Incubation of Su-9055 with rat liver mitochondrial or microsomal fractions with added $NADH_2$ or $NADPH_2$ did not yield any reduced Su-9055 in contrast to metopirone which, under identical conditions, was readily converted to its reduced form. Most of the Su-9055 was found to be unchanged and this excludes any possibility of protection through steric similarity of the reduced form of Su-9055 to 7-OHM-12-MBA, the active metabolite of DMBA. The protective action is more likely due to some other feature of the molecule as first proposed by WONG and WARNER[9] or a different mechanism.

Résumé. Nous avons réduit le Su-4885 et le Su-9055 pour étudier l'action de ces substances contre la destruction des adrénales par le DMBA. Les effets inhibiteurs ne peuvent pas être expliqués par les changements que le foie produit sur le métabolisme du DMBA.

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