hepatic glutathione reductase activity by the method of WENDELL[7]. Protein was determined by the method of LOWRY et al.[8] Assays were also performed with FAD added to the methemoglobin reductase assay in a final concentration of 1 μM and to the glutathione reductase assay in a final concentration of 4 μM.

Results and discussion. At the time of sacrifice, riboflavin deficiency was readily apparent in rats of Group 1, who showed rough coats and a failure to gain weight[9,10]. Weight-gains of rats receiving the deficient diet lagged significantly behind those of animals whose diets were sufficient in riboflavin ($p < 0.001$)[11]. Furthermore, riboflavin deficiency of Group 1 animals was corroborated biochemically by measuring the activity of hepatic glutathione reductase. The Table shows that activity of this enzyme of animals in the deficient group was significantly below that of animals receiving adequate riboflavin intake ($p < 0.001$). However, erythrocyte methemoglobin reductase activity was not affected by riboflavin deficiency (Table). Slight augmentation of enzyme activity was observed in all groups with addition of FAD in vitro, but the increases were insignificant. The above findings show that assay of erythrocyte methemoglobin reductase activity is not a valid method for detection of riboflavin deficiency.

Enzyme assayed	Groups[a]	Mean specific activity[b]	Standard error	t[c]	p[d]
Erythrocyte methemoglobin reductase	1	4.7	0.7	No significant difference	
	2	4.0	0.7		
	3	4.9	0.6		
	4	5.1	0.7		
Hepatic glutathione reductase	1	31.5	4.8	3.82	< 0.001
	2	50.2	4.5		
	3	57.7	5.9		
	4	50.5	2.8		

[a] Six rats in each group: 1. Riboflavin-deficient diet; 2. Deficient diet with riboflavin supplementation; 3. Regular diet with riboflavin supplementation; 4. Regular diet. [b] Activity of methemoglobin reductase is expressed as nmoles methemoglobin reduced/min/mg Hb, and activity of glutathione reductase is expressed as µmoles glutathione reduced/min/g protein. [c] t = Student's t-test for comparison of difference between means of enzyme activities of deficient and combined non-deficient groups. [d] p = p-value as determined by two-tailed test.

Zusammenfassung. Bei Ratten mit Riboflavin-Mangel wurde eine normale Aktivität der NADH-abhängigen Methämoglobinreduktase festgestellt.

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Side-Chain Hydroxylation in the Biosynthesis of β-Ecdysone (20-Hydroxyecdysone) in the Blowfly *Calliphora stygia*

From a study[1] of the metabolism of 25-deoxyecdysone in the blowfly *C. stygia* it was concluded that side-chain hydroxylation probably precedes modification of the steroid nucleus in the biosynthesis of β-edysone (20-hydroxyecdysone) from cholesterol. To test this conclusion we have now studied the metabolism in *C. stygia* of [26-^{14}C]-25-hydroxycholesterol and [22-^{3}H-]-(22R)-22-hydroxycholesterol.

A colloidal suspension of [26-^{14}C]-25-hydroxycholesterol (37.0×10^6 dpm, 60 mCi/mmole) in water containing 0.5% sodium oleate was injected into 3rd instar larvae of *C. stygia* (100 insects) 6–9 h prior to puparium formation together with [1-^{3}H]-cholesterol (145×10^6 dpm, 10 Ci/mmole) to serve as a reference standard (^{3}H:^{14}C = 3.9:1). The prepupae were collected 6–9 h after puparium formation (about 18 h incubation), homogenized in ethanol and the β-ecdysone isolated as described previously[2]. As observed earlier[3], [1-^{3}H]-cholesterol was found to be incorporated into β-ecdysone (0.016%). However, the ^{3}H:^{14}C ratio of the purified β-ecdysone isolated was 20:1, indicating that 25-hydroxycholesterol, if incorporated, was incorporated to a much lower extent than cholesterol. Recently it was reported[4] that 25-hydroxycholesterol is not metabolised to β-ecdysone in seedlings of *Podocarpus elatus*.

[22-^{3}H]-(22R)-22-hydroxycholesterol (1×10^9 dpm, 2.46 Ci/mmole, radiochemical purity 98%), prepared by reduction of 22-ketocholesterol with sodium borotritide[5] was incubated in *C. stygia* and the β-ecdysone isolated as before. After extensive purification the β-ecdysone fraction was chromatographed with non-radioactive β-ecdysone. The curve of the UV-absorption plotted against elution volume then coincided with a weak peak of radioactivity which corresponded to an incorporation of not more than 0.0001%, that is about 1/100 of that obtained with cholesterol.

The cholesterol pool in *C. stygia* is very large[3] and probably very much larger than any of its more polar

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metabolites[6]. It is thus clear that the amount of incorporation observed with the hydroxycholesterols it is not significant and that neither of these compounds can be biological precursors of β-ecdysone. By contrast is was found that in *C. stygia* 25-deoxyecdysone[1] and 22-deoxyecdysone[7] rapidly afforded β-ecdysone by side-chain hydroxylation. It appears that the enzymes responsible for the modification of the nucleus are sensitive to the status of the side-chain and it is likely that in the biosynthesis of β-ecdysone from cholesterol some elaboration of the 5-ene system of cholesterol must take place before hydroxylation of the side-chain can proceed[8].

Résumé. Dans la mouche a viande *Calliphora stygia* le 25-hydroxycholesterol et le (22R)-22-hydroxycholesterol sont incorporés a la β-ecdysone (20-hydroxy-ecdysone) beaucoup moins qu'au cholesterol et ne sont donc probablement pas des précurseurs de cette hormone.

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Cellular Proliferation in the Arterial Walls of Epinephrine-Treated Rabbits

Recent studies have shown that both in man and animals the smooth muscle cells of the arterial wall may actively proliferate in several pathological conditions. So far, the reports in this field have been mainly concerned with human and experimental atherosclerosis[1–6]; very little is known as to other arterial lesions[7,8]. As early as 1903, Josué[9], confirmed by others[10–13], reported the production of arterial lesions in rabbits after i.v. injections of epinephrine. The most characteristic features of this arteriopathy are necroses in the media of the large elastic arteries with an increase of PAS-stainable mucopolysaccharides, followed by calcium deposition. The aim of the present work was to study in light and electron microscopy the early stages of the epinephrine arteriopathy and to determine by ^{3}H-thymidine and colchicine methods whether after epinephrine the medial smooth muscle proliferates.

Adult rabbits weighing about 2 kg were used. 4 untreated animals served as control. 6 animals received i.v. a total dose of 500 μg/kg body wt. of epinephrine during a period of 12 h. Other 6 rabbits received i.v. twice daily 25 μg/kg of the drug. The animals of both treated groups were killed 1, 3, 5, 7, 10 and 12 days after the beginning of the experiment. 1 h before the sacrifice ^{3}H-thymidine, 1 mC/kg of body wt., was injected i.v. For colchicine studies 6 rabbits were injected with daily doses of 100 μg/kg of epinephrine and killed as above. Colchicine, 1 mg/kg body wt., was administered s.c. 9 h before the sacrifice. At autopsy, selected segments of the main pulmonary artery and of the aorta were processed partly for light and electron microscopy and partly for autoradiography. For light microscopy the conventional histological methods were employed. For electron microscopy the tissues were fixed in Karnowsky mixture and refixed in osmium tetroxide, embedded in Epon, subsequently stained with uranyl acetate and lead citrate (Reynolds) and examined under a Siemens Elmiskop IA electron microscope. For autoradiography the usual techniques were employed. In each arterial segment the mean number of labelled nuclei per mm^2 of the surface area of the media was calculated. In the untreated rabbits, ^{3}H-labelled nuclei were only exceptionally seen and colchicine mitoses (metaphases) were never observed.

In the rabbits treated with a single high dose of epinephrine, the histological picture was primarily one of involvement of the media. There was a separation or loosening of the medial elastic and muscle components due to the accumulation of basophilic metachromatic material. Later these elements appeared to be degenerated and necrotic. Electron microscopic examination showed widened intercellular spaces containing an electron-lucent, structureless material. Associate findings were necrotic or injured smooth muscle cells showing swollen mitochondria with disorganized cristae and electron-lucent matrix. Scattered vacuoles were frequently seen and most of the myofilaments appeared as granular debris. Regressive changes were found only in the rabbits killed up to 7 days after epinephrine; thereafter the normal structure appeared to be fully restored. As reported in the Table (left) small numbers of labelled nuclei were constantly found in the media of the aorta and pulmonary artery, independently from the time elapsed after treatment and even after apparent restoration of the arterial structure (Figure 1). Similarly treated animals injected with colchicine showed metaphase-arrested mitoses, mainly concentrated in the inner-

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