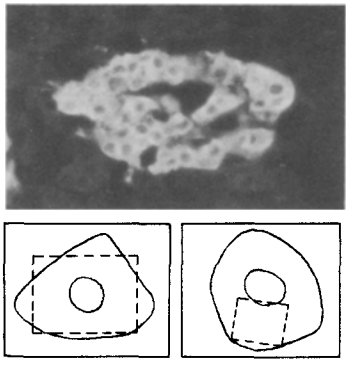

Figure 1. An immunofluorescence micrograph of the rat adrenal gland stained with anti-tyrosine hydroxylase antiserum. ×230. A large square of 11 × 15 μm is shown by a dotted line for dilution test of figure 2, and a small square of 6.6 × 7.3 μm by a dotted line for average fluorescence of the table.

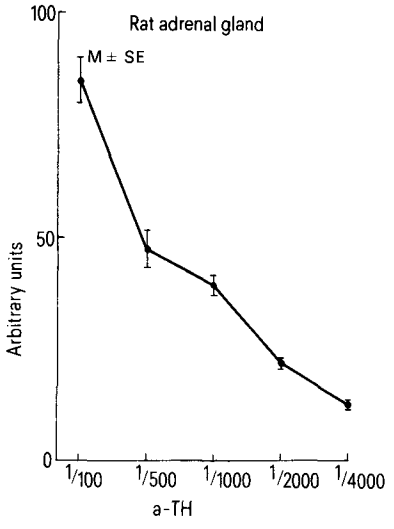

Figure 2. Change in fluorescence of the cells in the adrenal medulla of SHR exposed to dilutions of an anti-tyrosine hydroxylase antiserum. The measurement head delimited a square of 11 × 15 μm for a whole single cell including a nucleus. The sensitivity is 10 times lower than in the table.

for 30 min in a moistened chamber, specimens were rinsed as above. After air drying, glycerin/phosphate buffer was used for mounting.

A Leitz Dialux 20 fluorescence microscope with a MPV compact microscope photometer was used on the original section slide to give read-outs by printing. The standard light source for microscopic fluorimetry in incident light illumination was a XBO 75 W high-pressure Xenon lamp in a lamp housing 100 Z. All measurements were made with a single diagram setting in the measurement head delimiting a square of 11 × 15 μm including the nucleus (figs 1 and 2), or 6.6 × 7.3 μm excluding the nucleus (table) in the object plane for a single cell. Since the immunofluorescence faded slowly during excitation, the time of fluorescence measurements was made constant for 2.5 sec. and no more than one cell was measured per field.

Results. Figure 1 shows an example of a fluorescence micrograph of the rat adrenal gland stained with anti-TH antiserum. For the dilution test, the measurement head delimited a square of 11 × 15 μm for a whole single cell including a nucleus. The sensitivity used for measurement was 10 times lower than the table. For the comparison of average fluorescence of the adrenal medulla of SHR and WKY, the measurement head delimited a square of 6.6 × 7.3 μm for a single cell but excluding a nucleus. The sensitivity was 10 times higher than figure 2. Figure 2 shows the resulting plots of fluorescence brightness versus dilution of TH antiserum. Reduction in fluorescence was almost linear in the dilution range of 1:100–1:4000. At higher dilutions fluorescence levels approached base-line. In the experiments, measurements were made at a 1:500 dilution of TH antiserum only. Readings on single cells excluding nuclei were 100–800 against background values of 10–15. TH-immunoreactive fluorescence intensity in the cells of the adrenal medulla was compared between SHR and WKY at 4 weeks and 16 weeks of age. As shown in the table, TH-immunoreactive fluorescence intensity is higher in the cells of the adrenal medulla of SHR than in the cells of WKY both at 4 weeks of age and at 16 weeks of age. This result agrees with our previous reports[3,4] indicating increased TH activity in the adrenals of SHR, and suggests that the amount of TH protein in the adrenal medulla is increased in SHR.

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The egg-lipid composition of the 'living fossil' reptile tuatara *(Sphenodon punctatus)*

D.R. Body

Applied Biochemistry Division, Department of Scientific and Industrial Research, Palmerston North (New Zealand), 3 July 1984

Summary. The lipid composition of two tuatara eggs was examined. The eggs contained triacylglycerol (80%) and phospholipid (12%) as their major lipid fractions. Fatty acid analyses of the individual lipid classes indicated the presence of essential fatty acids, linoleic and arachidonic acids. The quantity of such acids in the egg yolk lipids would suggest they are factors for survival as illustrated in other species.

Key words. Tuatara; *Sphenodon punctatus;* egg composition; egg yolk lipids.

The tuatara *(Sphenodon punctatus)* is the only known survivor of the ancient rhynchocephalian order of reptiles[2]. Since the Mesozoic period this species has only been located in New Zealand where its survival was probably due to the absence of terrestrial mammals. It was distributed throughout the country until the disruptive settlement of man caused its extinction on the mainland. These reptiles now only occur on certain islands off the coast of New Zealand and are rigidly protected by law[3]. The similarity of the skeleton and dentition pattern between the primitive *Sphenodon punctatus* and the well studied European Sphenodontid fossils indicates there has been very little change in the anatomy of the tuatara over this period of time[2]. Recent work has been carried out on the structure of the yolk[4] and shells[5] of the tuatara eggs and these have been compared with other vertebrates. However little biochemical information appears to be available. This paper reports on the lipid composition of tuatara eggs and their corresponding fatty acid complement.

Materials and methods. Two tuatara eggs were obtained from Stephens Island (near Wellington). The newly laid egg was white in color whilst the other was brown and estimated to have been incubated for over 10 months. These were aged according to recognized criteria[5] and were confirmed on examination of the embryonic stage.

Lipid extraction. Tuatara eggs have parchment-like shells that can be readily cut open with sharp scissors. The egg contents (yolk and albumen) were removed from the shells which were washed, dried and weighed. The total egg contents (after the embryo was removed) were extracted with $CHCl_3$-MeOH (2:1 v/v) according to the method of Folch et al.[6] to provide fractions of total lipid extract and crude protein residues. The lipids were fractionated by silicic acid column chromatography monitored by TLC[7]. The acyl moieties of the recovered triacylglycerol and phospholipid fractions were converted into methyl esters by direct transesterification[8]. Any unesterified fatty acid fractions were methylated using diazomethane[9]. All fatty acid methyl esters were analyzed by GLC under the conditions outlined elsewhere[10].

Results and discussion. The characteristics of the eggs are shown in table 1. The differences observed between the wet weight of these eggs could be due to the expected overall weight differences within clutches of eggs, and variations related to the exchange of water between the contents of the egg and the environment. Such changes apply particularly to parchment shelled eggs[11]. This process, together with associated biological features, have been reviewed recently by Tracy[12].

From the total organic matter isolated from the tuatara eggs, it was found that the weight ratio between the lipid to protein contents was similar, e.g. egg 1 (1.0:1.2) and egg 2 (1.0:1.3). As compared with the domestic hen ratio 1.0:1.0[7] the tuatara eggs appeared to contain higher levels of protein matter. The quantity of lipid related to the total egg weight was higher in the newly laid tuatara egg (20.1%) than in the incubated egg (7.4%). Similar reductions occurred in the corresponding protein levels which suggest both lipid and protein resources were utilized for embryonic development over the incubation period of 10 months.

It is generally accepted that the rate of lipid metabolism in reptiles is slow when compared with birds and mammals[13]. More specifically it has been reported that the lipogenic capacity of tuatara adipose tissue is less than those of more modern reptiles[14]. This information may explain why less yolk-lipid (as the energy reserve) is required for the development of the tuatara embryo. However, on examining the tuatara egg lipids (table 2), the content of lipid energy storage, i.e. triacylglycerol at 77.9 to 81.6%, was higher than that of the domestic hen (65%)[7].

The fatty acid composition of the tuatara egg lipids included substantial levels of essential fatty acids, such as linoleic and arachidonic acids in both neutral and polar lipid fractions, for subsequent embryo development (table 3). The presence of essential fatty acids in the egg lipids is a direct reflection on their availability as part of the diet of adult females, which in birds[7] improves the survival rate of hatched progeny.

Substantial levels of unesterified fatty acids were noted only in the incubated (egg 2) lipids (tables 2 and 3). Since the free fatty acid profile was different to the esterified fatty acid content of triacylglycerol and phospholipids, they may represent part of the fatty acid complement required for embryo development rather than an accumulation of hydrolyzed products of the parent lipid released during sample preparation.

Other aspects of tuatara history have been documented[2]. Since few changes have been recorded in their anatomy, as compared

Table 1. Contents of tuatara eggs

Material	Egg 1	Egg 2
Wet weight (g)	3.266	5.908
Lipid extract (mg)	655.0	439.5
Protein content (mg)	791.7	570.7
Shell (mg)	282.7	343.2

These eggs represent two stages of incubation, egg 1 (zero) and egg 2 (10 months).

Table 2. The lipid composition of tuatara eggs

Components	Egg 1	Egg 2
Cholesteryl esters	2.0	1.2
Triacylglycerols	81.6	77.9
Fatty acids	tr[a]	3.5
Cholesterol	2.6	2.8
Phospholipids	13.8	12.5
Unidentified	–	2.1

These eggs represent two stages of incubation, egg 1 (zero) and egg 2 (10 months). The results are expressed as weight percentage of the recoverable lipid extracts.
[a] tr, trace less than 0.1%.

Table 3. Fatty acid composition of tuatara egg lipids

Fatty acids[a]	Egg 1		Egg 2		
	TG[b]	PL[b]	TG	FA[b]	PL
n-Saturated					
14:0	1.2	0.2	1.5	2.0	0.3
15:0	0.2	0.1	0.3	0.5	0.2
16:0	16.1	20.4	14.3	22.2	17.1
17:0	0.3	0.4	0.9	1.0	0.6
18:0	2.0	5.8	2.8	4.7	5.3
> 18.0	tr[b]	0.5	0.3	0.1	0.5
n-Unsaturated					
14:1	0.5	0.1	0.9	0.9	0.2
15:1	0.1	0.1	0.3	0.4	0.2
16:1	6.9	2.3	7.2	7.4	2.9
17:1	0.5	0.4	0.7	0.7	0.4
18:1	34.9	20.6	42.7	40.3	25.7
18:2	19.3	22.6	13.3	9.9	16.6
18:3	8.3	4.2	6.6	5.1	3.2
20:2	0.1	0.1	0.2	0.1	0.1
20:4	3.5	12.2	2.9	2.0	12.2
20:5	1.5	6.0	1.7	0.8	7.4
22:4	0.7	tr	0.6	0.4	1.2
22:5	2.2	1.9	1.8	1.1	3.4
22:6	1.7	1.2	1.0	0.4	2.5

These eggs represent two stages of incubation, egg 1 (zero) and egg 2 (10 months). These findings are expressed as percentage of the total fatty acid content. [a] Designation – carbon number: degree of unsaturation. [b] Abbreviations: TG, triacylglycerol; FA, unesterified fatty acids; PL, phospholipids; tr, trace, less than 0.1%.

with 'related' reptile fossils, one could speculate that in the light of current knowledge of tuatara egg lipids and the corresponding slow lipid metabolic activities[14], these findings may help explain the survival of the tuatara over such a long period of time.

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Acetylcholine induced endothelial-dependent vasodilation increases as artery diameter decreases in the rabbit ear

M.P. Owen and J.A. Bevan*

Department of Pharmacology, University of Vermont, College of Medicine, Given Medical Building, Burlington (Vermont 05405, USA), 18 June 1984

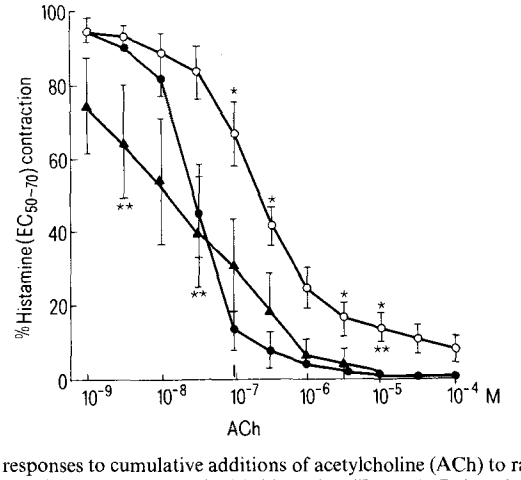
Summary. Isolated resistance vessels in the rabbit ear preconstricted with histamine were relaxed by acetylcholine by a proportionately greater amount than the central ear artery. The relaxation was antagonized by atropine and also by endothelium removal. Our studies represent the first direct evidence that endothelium-dependent dilation can occur in resistance vessels.
Key words. Acetylcholine; endothelium-dependent vasodilation; resistance vessel.

Furchgott and Zawadzki[1] discovered that the relaxation by ACh of isolated preparations of rabbit thoracic aorta and several other large and medium sized precontracted arteries is dependent on the presence of endothelial cells. Presumably because in vitro experiments using smaller vessels are technically difficult, no studies have appeared which analyze this phenomenon in resistance arteries. The aim of this study was to determine whether vasodilation of resistance vessels in the rabbit ear by ACh is endothelium-dependent and whether the dilation is quantitatively and qualitatively similar.

Male New Zealand White rabbits (2–3 kg) were stunned and exsanguinated. The central ear artery (CEA) (unstretched lumen diameter (ULD) ~ 300 μm), a main side branch (MSB) (ULD ~ 150 μm) of the CEA, and a terminal branch (TB) (ULD ~ 75 μm) of the MSB were removed and ring segments prepared. CEA segments were mounted on a standard tissue bath myograph[3]. The two smaller vessels were mounted on a smaller myograph using a modification[2] of a method used by Mulvany and Halpern[4]. Detailed procedures for isometric contraction and relaxation have been described in an earlier report[2].

Exogenous ACh caused a concentration-dependent relaxation of all arterial segments when they were initially constricted with histamine (H) (EC_{50}–EC_{70}) (fig.). Log IC_{50} values and the maximum relaxation produced by ACh at each concentration for the three vessels were compared by a one-way analysis of variance. Individual mean comparisons were performed using a multiple t-test with adjustment for multiple comparisons. $p < 0.05$ was accepted as a significant difference. By the criteria of IC_{50} value comparison, the TB was determined to be more sensitive to ACh than the CEA. The maximum relaxation produced by ACh did not differ significantly between the arteries (CEA, 92%; MSB, TB, 100%). The responsiveness to exogenous ACh was different in the three arteries as exhibited by significant differences in the concentration-effect curves (fig.).

The endothelium-dependency of the action of ACh was demonstrated in the CEA and MSB by the abolition of the relaxation response by mechanical removal of the endothelial layer (the arteries were rotated on their supporting wires). Rotated preparations of TB exhibited some relaxation of tone upon ACh addition – they relaxed 56–100% of initial relaxation. However,



Mean responses to cumulative additions of acetylcholine (ACh) to rabbit ear vessels initially constricted with histamine (EC_{50-70}). Relaxation responses are illustrated as percentage of the histamine contraction remaining upon acetylcholine addition in each experiment. Each point represents the mean ± SEM of different vascular segments (central ear artery (CEA) (○): n = 8; main side branch (MSB) (●): n = 4; terminal branch TB) (▲): n = 4). *Significant difference from MSB. **Significant difference from CEA.